

# **Screening for Chronic Kidney Disease (CKD) in a high risk population using a Point of Care Instrument for creatinine measurement: A community based study (The Bellville South Africa Study)**

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## **DECLARATION**

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**ABSTRACT:**

Chronic kidney disease (CKD) is described as abnormal kidney function in which one third is lost over a period of 3 months and is a global epidemic with a particularly concentrated incidence within developing countries, such as Sub-Sahara Africa (SSA). Health facilities in SSA are limited due to lack of funding and a dearth in disease and medical knowledge. This coupled with the high incidence of both communicable and non-communicable diseases makes for an ideal environment for the implementation of Point-of-Care Testing (POCT), defined as an analytical test that is performed near the patient, delivering results in real time without the need for a conventional laboratory. CKD POCT involves the measurement of creatinine in capillary whole blood samples in order to determine the estimated glomerular filtration rate (eGFR) of patients in order to stage their CKD status from stage 1-6.

This study aimed to bridge the gap in knowledge with regard to cut-offs of creatinine levels and eGFR values when screening a mixed ancestry populations. Currently there is only documented and standardized cut-offs for Caucasian and African American populations. This study looked at the African mixed ancestry population and acts as a starting point for standardizing POCT cut-offs for other international mixed ancestry populations.

103 participants were recruited from the Bellville South community, Cape Town, South Africa. The study was a comparative study that was designed to evaluate the Nova Statsensor® point of care instrument for the measurement of creatinine for the detection of CKD in adult mixed ancestry subjects from the Bellville South Community in South Africa. Secondary objectives included (1) the prevalence of CKD based on the results of the instrument, and (2) the correlation between the Nova Statsensor®, and the central laboratory creatinine values (IDMS traceable). Ancillary objectives of the study were to evaluate the technical quality of POC testing for creatinine in a community setting, as well as the evaluation of the cost implications when introducing this form of POCT into a primary care setting.

The study found that the Nova Statsensor® in this study had a sensitivity of 66.7% and a specificity of 100%, displaying excellent diagnostic accuracy. It was found that the device displayed negative proportional bias which may lead to future CKD patients being misdiagnosed as healthy within screening programmes. The prevalence was found to be 2.9% within this mixed ancestry population.

The device was user friendly and requires a small sample volume, however it is costly to implement.

The laboratory evaluation study found that the Nova Statsensor® creatinine meter produced a direct creatinine concentration comparison that was less than expected, possibly due to creatinine levels depending on several factors which include muscle mass, obesity, gender, and age and having a wide reference interval. Thus highlighting the importance of the use of the equations to calculate eGFR in CKD screening in order to obtain the CKD staging results which displayed better correlation to the reference method, compared to creatinine measurement alone. The device was comparable to the reference method when performance was measured based on CKD staging through the calculation of the MDRD equation.

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**LIST OF ABBREVIATIONS:**

ACR: Albumin/creatinine ratio

ADA: American Diabetes Association

ARV: Anti-retroviral

CG: Cockcroft-Gault

CKD: Chronic kidney disease

CKD-EP: Chronic kidney disease – Epidemiology Collaboration

CLSI: Clinical Laboratory Standards Institute

CP: Cell phone

CVD: Cardiovascular disease

eGFR: Estimated glomerular filtration rate

ELISA: Enzyme Linked Immunosorbent Assay

EQC: External quality control

ESRF: End-stage renal failure

GFR: Glomerular filtration rate

HAART: Highly Active Anti-retroviral Therapy

HbA1c: Glycated haemoglobin

HIV: Human Immunodeficiency Virus

HPLC: High Performance Liquid Chromatography

ICU: Intensive Care Unit

IDMS: Isotope Dilution Mass Spectrometry

IDT: Interdigital transducer

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

IQC: Internal quality control

ISO: International Standards Organisation

KDIGO: Kidney Disease – Improving Global Outcomes

KEEP: Kidney Early Evaluation Program

LAMP: Loop Mediated Isothermal Amplification

LOC: Lab-on-chip

LW-SAW: Love Wave - Surface Acoustic Wave

MDRD: Modification of Diet in Renal Disease

MEMS: Micro-Electro-Mechanical Systems

MTP: Microfluidics Tissue Processor

NKF: National Kidney Foundation

NPV: Negative predictive value

PBA: Paper-based assays

POCT: Point-of-Care Testing

PPV: Positive predictive value

QC: Quality control

QCM: Quartz crystal microbalance

RRT: Renal replacement therapy

SAW: Surface acoustic wave

SCr: Serum creatinine

T2DM: Type 2 diabetes mellitus

TB: Tuberculosis

TEa: Total allowable error

# **CHAPTER 1**

## **Literature Review & Project Overview**

## 1.1 INTRODUCTION

Chronic kidney disease (CKD) is a global epidemic with a particularly concentrated incidence within developing countries, such as Sub-Sahara Africa (SSA). Health facilities in SSA are limited due to lack of funding and limited medical care. This coupled with the high incidence of both communicable and non-communicable diseases makes for an ideal environment for the implementation of Point-of-Care Testing (POCT). POCT involves devices that range from hand-held to bench top machines that deliver test results within minutes, using less invasive sample collection methods and small sample volumes. Many of these devices make use of reagents and consumables that can be stored for long periods of time at room temperature therefore making them ideal for rural regions. They are relatively easy to use and do not require much training.

However current POCT devices' cut off limits are generally standardized for Caucasian and African American peoples. This presents a question with regard to the devices' accuracy when testing a mixed ancestry population, as is found in SSA and South Africa. (NIH: estimating glomerular filtration rate)

## 1.2 CHRONIC KIDNEY DISEASE

### 1.2.1 Background

Chronic kidney disease is described as abnormal kidney function in which one third of kidney function is lost over a period of 3 months (Johnson *et al*, 2004). CKD consists of 6 stages (Table 1.1) which are classified based on the estimated glomerular filtration rate (eGFR). (NHS Horizon Scan Report, 2014). CKD often goes undetected until it has reached stage 4 or 5. Once stage 5 has been reached, the patient enters End-Stage Renal Failure (ESRF) which requires renal dialysis or even a kidney transplant, if there is an available donor. These treatments are extremely costly to both the patient and state health budgets. It has been estimated that over 1.4 million people worldwide receive renal dialysis, and the incidence has been found to be increasing by 8% annually.

**Table 1.1:** CKD stages and their related eGFR values.

| CKD Stage | eGFR (mL/min/1.73m <sup>2</sup> ) | Kidney function                  |
|-----------|-----------------------------------|----------------------------------|
| 1         | >90                               | Normal or high                   |
| 2         | 60-89                             | Mildly decreased*                |
| 3a        | 45-59                             | Mildly to moderately decreased   |
| 3b        | 30-44                             | Moderately to severely decreased |
| 4         | 15-29                             | Severely decreased               |
| 5         | <15                               | Kidney failure                   |

\*Usually young adults

CKD is also often linked to several other non-communicable diseases; e.g. diabetes mellitus (Warram *et al*, 1996), hypertension (Schulman *et al*, 1989), and cardiovascular disease (CVD) (Angelantonio *et al*, 2010). CKD develops in one third of diabetic patients and in one fifth of patients suffering from hypertension. It has been estimated that CKD is associated with premature mortality from CVD. The risk of death by CVD is increased by 46% in people who have an estimated glomerular filtration rate (eGFR) between 30 and 60 mL/min per 1.73 m<sup>2</sup> (Matsha *et al*, 2013). These findings were independent of traditional cardiovascular risk factors, which include diabetes mellitus and hypertension. (Johnson *et al*, 2004). According to the KDIGO, 2012, hypertension is responsible for over a quarter of the world's rate of kidney failure, and diabetes about one-third of the world's kidney failure rate.

In a study conducted by Wali (2010), it was estimated approximately 500 million individuals have CKD globally. This translates to 1 in 10 adults globally. CKD is a growing epidemic and is putting financial strain on government healthcare systems and developing countries are struggling to cope with this burden. In China, over the next decade, the economy will suffer a 558 billion US dollar loss due to heart disease and kidney disease deaths and medical treatment. Uruguay is currently experiencing an annual cost of 23 million US dollars, 23% of its National Resources Fund budget, just on the treatment of dialysis (KDIGO, 2012).

Over 2 million people globally receive dialysis treatment or kidney transplants in order to survive kidney disease. Of this 2 million, only 20% are treated in over one hundred developing countries such as: South Asia, India, and SSA, compared to the nearly 80% treated in five developed countries: the United States, Japan, Germany, Brazil, and Italy (National Kidney Foundation: <https://www.kidney.org/kidneydisease/global-facts-about-kidney-disease>). To put these statistics into perspective one must note that the five developed countries only make up 12% of the world's population, whereas the one hundred developing countries make up over 50% of the global population. Recent estimates have demonstrated that SSA contributes less than 4000 ESRD dialysis patients to the global dialysis population. This is less than 1% of the total global dialysis population (Bamgboye 2013). This illustrates the poor health care and limited resources within developing countries which sadly results in many preventable deaths (KDIGO, 2012). In 112 developing countries, it has been found that there are over 1 million deaths annually due to citizens being unable to afford kidney disease treatment (KDIGO, 2012).

The exact magnitude of CKD in SSA countries is still poorly characterized, however it is speculated that the incidence rates are 3-4 times higher than those in developed countries (Naicker S. 2009). Available data on CKD in Africa has been largely derived from hospital-based studies which were carried out in tertiary care facilities. There have been very few community-based studies. The reported high fatality rate associated with CKD in Africa has been attributed to several factors which include the increasing prevalence of communicable diseases such as AIDS, late referrals of CKD sufferers to a specialist, poor prognosis, limited renal replacement therapy, and lack of health care workers trained in kidney disease prevention (Arogundade & Barsoum. 2008).

Jha et al (2013) found that CKD affects mainly young adults ranging 20-50 years of age, in SSA. The main causes being hypertension and glomerular diseases which arise from inadequate treatment due to late referral to a health care facility. A noteworthy communicable disease found to be associated with nephrology in SSA is Malaria. Malarial (*Plasmodium falciparum*) nephropathy results in fluid and electrolyte disorders, glomerulonephritis, and in some cases acute renal failure (Eiam-Ong S, 2003).

It was also found that SSA greatly lacked renal replacement therapy due to limited availability and frightfully high costs. Funding for renal replacement therapy was found to be funded mostly through private organisations, with the government contributing minimally and to a very limited number of patients. These factors all contribute to the high mortality rate from CKD in SSA. Furthermore, in many African countries there is limited or even no access to renal replacement therapy (RRT) and therefore again results in many preventable deaths (KDIGO 2012).

Although CKD preventative measures have been implemented in Africa, they are still in their early infancy and thus do not have a large enough impact yet to see a decline in CKD prevalence and mortality. Another attributing factor is the lack of standardisation of CKD diagnostic tests which are currently in use.



Screening programs, in areas predetermined to be at high-risk for developing CKD, have been introduced to some parts of Africa. These programs involve identifying populations with a high prevalence of non-communicable diseases such as diabetes mellitus, cardiovascular disease (CVD), and hypertension. Once identified, they are screened for CKD based on their blood creatinine levels in order to determine their eGFR. A patient's urine can also be tested for albumin to determine CKD through the Albumin/Creatinine ratio (ARC) (Mattix *et al*, 2002). Elevated levels of urinary albumin excretion in patients suffering from diabetes mellitus are an early warning sign that the patient is heading towards diabetic nephropathy. Clinically significant nephropathy is diagnosed when a patient presents with urinary protein excretion that exceeds 500 mg/24 h (Hutchison *et al*, 1988). The conventional timed urine sample collection for microalbuminuria diagnosis, using the albumin excretion rate method, has proved clinically tedious and impractical, due to the incomplete sample collection caused by missed void times. This timed method also requires a lot of time input from both the patient and doctors involved (Warram *et al*, 1996). These challenges gave rise to the development of the albumin/creatinine ratio (ACR) measurement. This form of measurement involves urine sample collection at random void times. It was proposed that screening for microalbuminuria using the ACR value of  $\geq 30$   $\mu\text{g}/\text{mg}$  as abnormal (Warram *et al*, 1996). The untimed and random sample collection method used in the ACR method are advantageous to the patient as the sample is small and can be transported in a handbag or pocket making it unnecessary for the patient to remain at the hospital all day. This method of collection also makes strict, accurate timed sample collection unnecessary, which is much easier for patients to follow (Hutchison *et al*, 1988). A study conducted by Warram *et al* (1996) found that there was a very close relationship between the ACR method, with random timed collection points, and the albumin excretion rate method, with a 3 hour urine collection immediately after a random void, having an  $r^2$  value of 0.94. From these findings it was concluded that the ACR method can be used to diagnose early stages of diabetic nephropathy in diabetic patients (Warram *et al*, 1996).

Screening for diabetes mellitus involves either the measurement of the HbA1C, also known as glycated haemoglobin, or the measurement of the blood glucose levels. Hypertension is simply screened for by taking a measurement of the patient's blood pressure. Screening programs often utilise POCT instruments as such areas in Africa often lack conventional laboratories.

If we are to make any significant progress with regard to decreasing the prevalence of CKD and other related non-communicable diseases within Africa, we need to make an effort to ensure that both patients and health care workers are educated about diabetes mellitus, hypertension, CVD, and CKD. This will ensure that they are aware of the signs and symptoms, possible risks, and ways in which to manage and even prevent the onset of such diseases (Naicker. 2009).

### 1.2.2 CKD Symptoms

During the early stages of CKD many people remain asymptomatic and therefore do not seek medical attention until the disease has significantly progressed. CKD presents several non-specific symptoms that are not too alarming at first to patients. Several examples include: increased fatigue, decrease in appetite and weight-loss, difficulty in concentrating, insomnia, night-time muscle cramping, swollen ankles and feet, skin irritation, increased night-time urination, nausea, hypertension, and puffiness surrounding their eyes. Many patients only suffer from a few of these symptoms and think nothing of it, until the CKD progresses and presents more symptoms which causes concern (Levey *et al*, 2003).

Patients are also likely to observe blood within their urine. Through the use of a rapid dipstick test medical practitioners are able to detect any proteinuria in the urine. The biggest alert to decreased kidney function is the presence of albumin within a patient's urine. (Berns, 2016)

### 1.2.3 CKD Risk Factors

#### 1.2.3.1 Non-communicable diseases

##### i) Diabetes:

According to the American Diabetes Association (ADA) 2013, diabetes mellitus is “a group of metabolic diseases characterized by hyperglycaemia, resulting from defects in insulin secretion, insulin action, or both”.

It was found that 9.4 million people in Africa suffer from diabetes mellitus and predicted an estimated 140 % increase in diabetic patients by 2025, which translates to 12.7 million new cases of diabetes mellitus within Africa alone (Naicker. 2009). The International Diabetes Federation stated that in 2015 there were 415 million diabetes mellitus sufferers. They projected that this statistic will rise to 642 million sufferers by the year 2040 (Naicker. 2009).

According to Hjelmessaeth, *et al.* (2010), obesity and insulin resistance are the two most common causes for the development of type 2 diabetes mellitus (T2DM). Muscle plays a very important role in controlling the body's insulin levels as skeletal muscle is the most important site of insulin resistance. Skeletal muscle also accounts for glucose disposal after infusion. Due to this, it has been found that muscle mass is inversely proportional to insulin resistance. Conversely, creatinine is the only metabolite of creatine, which is primarily found in striated muscle. Low serum creatinine levels has been linked to an increased risk of developing T2DM (Harita *et al*, 2009). A study conducted by Harita *et al* (2009), which was carried out in non-obese middle aged Japanese males, found this exact relationship thus leading to the conclusion that low creatinine levels can also indicate a low muscle mass with little risk of developing T2DM, however this was only applied to non-obese subjects. An obese subject with low muscle mass and subsequently low creatinine levels would be considered to be at high risk of developing T2DM (Harita *et al*, 2009).

Another study conducted by Warram, *et al* (1996), found that type 1 diabetic patients (T1DM) with elevated levels of albumin, within their urine, were most likely to develop diabetic nephropathy.

From the above, one can deduce that both T1DM and T2DM patients carry a significant risk of developing either chronic kidney disease or end-stage renal failure. Pre-diabetic patients can avoid developing kidney disease by modifying their diet and enforcing weight loss, if overweight or obese.

The highest prevalence of diabetes is found in those of Indian origin, with the next highest being found in black population. Caucasians have been found to have the lowest prevalence compared to the above two populations (Mbanya & Ramiaya, 2006) It was found that the prevalence of diabetes in black populations followed a westernization gradient.

According to the ADA (2013), symptoms of diabetes include polyuria, polydipsia, weight loss, and blurred vision. Hypertension nearly always accompanies diabetes. Acute life threatening uncontrolled diabetes presents with hyperglycaemia with ketoacidosis in T1DM sufferers. Long term complications of diabetes include retinopathy, nephropathy, and peripheral and autonomic neuropathy.

As previously mentioned, there are two types of diabetes: T1DM and T2DM. T1DM involves complete deficiency of insulin production and secretion with  $\beta$ -cell destruction (ADA, 2010). Treatment involves intensive exogenous insulin administration with  $\geq 3$  injections per day, or insulin pump therapy or continuous sub-cutaneous insulin infusion (ADA, 2010). T2DM involves a combination of resistance to insulin action and an inadequate insulin secretory compensation response. Treatment entails glycaemic control through diet and exercise, or alternatively the addition of oral glucose lowering medication such as metformin drugs (ADA, 2010). T2DM does not require exogenous insulin (ADA, 2013)

According to the ADA 2010 guidelines, diabetes diagnosis involves one of several tests. Such tests include the Oral Glucose Tolerance Test (OGTT), ketone measurement, and the Fasting Plasma Glucose (FPG) test. Screening for diabetes makes use of HbA1c tests. The OGTT is supported by the WHO, but Sacks *et al* (2002) rejected it as a routine test because prominent limitations such as poor reproducibility. WHO supporters, however, argue that the OGTT has a higher sensitivity in diagnosis compared to the FPG test. The OGTT is currently the gold standard method for diabetes and pre-diabetes classification (NHANES OGTT procedures manual, 2007). The OGTT requires a patient to fast for 9-hours prior to taking the test. Upon commencement of the test, venous blood is first collected then a sugar drink is administered for oral ingestion. After 2 hours another venous blood sample is collected in order to examine the body's response to the ingested sugar (NHANES OGTT procedures manual, 2007). The cut-off for diabetes diagnosis is a blood plasma glucose level of  $\geq 11.1$  mmol/L. The 2-hour plasma glucose 75g OGTT includes diagnosis of Impaired Glucose Tolerance (IGT) which is diagnosed at a concentration range of 7.8 – 11.0 mmol/L. Results within this range is considered too high to be normal but too low to be considered as fully diabetic and are thus termed "pre-diabetic" (ADA, 2013).

There are however several limitations to the OGTT. Such limitations include: uncertainty when it comes to patient adherence to the required fasting period, a poor reproducibility of the 2-hour glucose tests, the OGTT is time consuming, and there is poor agreement between the FPG and 2-hour plasma glucose levels. (Zhou *et al*, 2010)

These limitations have paved the way forward for the development and standardization of HbA1c testing methods.

Another form of diabetes diagnosis testing is through the Fasting Plasma Glucose (FPG) test which is used to diagnose both diabetic patients and Impaired Fasting Glucose (IFG) pre-diabetic patients. Again this involves blood sampling however it does not require fasting for the casual FPG cut off limit of 11.1 mmol/L. If fasting was adhered to then the FGP cut off limit is lowered to 7.0 mmol/L. IFG is diagnosed when glucose concentrations fall within the range of 5.6 – 6.9 mmol/L.

The above two methods of testing are not reliable for patients suffering from any form of abnormal red blood cell (RBC) turnover illness such as haemolytic anaemia.

If any of the above test methods yield positive results, it is required that retesting be carried out the following day. If still positive then a sample must be sent in an accredited laboratory for confirmatory testing using the gold standard test method, which must also be re-run if found positive. However if the patient presents with clinical symptoms such as classic glycaemia then re-testing is unnecessary. (ADA, 2010)

HbA1c is an additional form of diabetes detection. It is best used as a screening tool and method of disease monitoring as it does not suffice as a diagnosis method (ADA, 2010). HbA1c measurement reflects an average of glucose levels over a 2-3 month period. According to the ADA (2013), HbA1c assays are currently highly standardized with a “diagnosis” cut-off of  $\geq 6.5\%$ . This established cut-off is associated with the inflection point of retinopathy prevalence and has been found to correlate with the FGP and OGTT diagnostic cut-offs (ADA, 2010). HbA1c is a quick, minimally invasive “screening diagnosis” test which can be self-measured by patients at home. However a legitimate diagnosis requires measurement in an accredited laboratory (ADA, 2010)

Diabetes often damages the kidneys and their filtering abilities. High blood glucose levels can result in the kidneys filtering too much blood at a time, thus putting the kidney tubules under excessive stress. Over time this results in the kidneys leaking extra protein, known as albumin, into the urine resulting in microalbuminuria (ADA: Kidney disease – Nephropathy). The loss of kidney filtering efficiency also results in a build-up of waste products within the blood. These factors in time cause CKD which then can lead to ESRD. (ADA: Kidney disease – Nephropathy)

According to Mbanya and Ramiya (2010), diagnoses should also take into account the presence of other diabetes risk factors. These include: Age, ethnicity, family history, and level of physical activity.

#### **ii) Hypertension:**

Hypertension has a negative impact on many organs, primarily the kidneys. Most kidney diseases, including CKD, are associated with increased blood pressure. It has been found that hypertension directly causes a decrease in kidney filtration and indirectly decreases perfusion. These effects result in an increase in creatinine levels, indicative of kidney disease.

Hypertension affects the blood vessels, resulting in decreased blood flow, depriving vital organs of oxygen. Hypertension damages the kidneys by damaging the nephrons within the kidney causing decreased kidney function. As excess fluids and waste products cannot be adequately filtered out and excreted the blood pressure further increases (Shulman et al, 1989).

According to Jha et al (2013) the global prevalence of hypertension in the year 2000 was 972 million cases, with most cases being found in developing countries. It has been estimated that this figure will rise to 1.56 billion cases by the year 2025. These projections have taken into account age-specific and sex-specific adjustments (Jha et al, 2013).

#### **iii) Cardiovascular disease (CVD):**

It has been found that CVD is closely associated with the progression of CKD to end-stage renal failure (Vanholder *et al*, 2005). Patients with any form of kidney dysfunction should receive immediate and adequate preventative care against CVD, therefore decreasing the chance of cardiac episodes.

Weiner *et al* (2004) stated that patients receiving dialysis treatment are 10 to 30 times more likely to die from CVD events. This study reviewed previous studies and pooled together the data in order to determine what risk CKD poses for those at risk of CVD. It was speculated that CKD increased the risk of CVD outcomes via several manners; the degree

of reduced kidney function can act as an indicator for both duration and severity of other CVD contributing factors (for example: hypertension), and also reduced kidney function could exacerbate any underlying CVD risk factors such as hypertension and cardiomyopathy. 18.4% of the study population were of African American descent with the rest being Caucasian. Weiner *et al* (2004) concluded that CKD was an independent risk factor with a hazard ratio of 1.19, and there was a significant relationship between kidney function and race. African American (black) individuals had a hazard ratio of 1.76 while Caucasians (whites) had a hazard ratio of only 1.13. These results suggest that CKD is a risk factor all-cause mortality and CVD in the general population, while at the same time being a more pronounced risk factor in African Americans rather than Caucasians.

#### **iv) Obesity:**

The prevalence of obesity is rising at an alarming rate in the adult population, but even more alarming is the rise of overweight and obese children worldwide. Jha *et al* (2013) found that from the year 2000 there were an estimated 312 million obese adults globally. Interestingly enough, it was found that in developing countries there is a steady rise in obesity, however this is concentrated in the affluent and educated regions of said developing countries.

As previously mentioned, the study conducted by Harita *et al* (2009) found that there is an inversely proportional relationship between muscle mass and insulin resistance. This can be related to obesity as a risk factor for CKD because obese people lack healthy levels of skeletal muscle and therefore tend to experience increased levels of insulin resistance. This leads to T2DM which as previously explained is a major risk factor for developing CKD.

Obesity also often leads to hypertension which can lead to renal damage and subsequent development of CKD.

From the above relationships between CKD and the fore mentioned NCD's one can conclude that obesity is an indirect cause of CKD. This is because obesity results in other NCD's that directly result in the development of CKD.

Adipose tissue is an endocrine organ, highly active, which secretes many factors that aid in the development of renal and cardiovascular complications (Ruster & Wolf, 2013).

There are many adipokines which are involved in causing renal injury. These work through controlling endothelial dysfunction and inducing oxidative stress and inflammation. Adipokines have also been found to be linked to renal anaemia (Ruster & Wolf, 2013). There is a delicate balance between the different adipokines, such as protective adipokines and those mediating pathophysiological effects. If this balance is disrupted, as caused by obesity, renal damage is initiated. Such damage can be contained and in some cases reversed by simply a change of lifestyle. One involving exercise and a balanced diet (Ruster & Wolf, 2013). Additional options for combating renal inflammation include pharmacological agents, and as a last resort; dialysis (Akchurin & Kaskel, 2015).

Akchurin and Kaskel (2015) stated that it has been clearly shown that an inverse relationship between eGFR and inflammation exists. However the roles of individual cytokines in CKD and ESRD are still being investigated.



Most recently, a lot of focus has been placed on the role of adipokines in CKD; specifically pro-inflammatory adipokines such as leptin, resistin, and the anti-inflammatory adiponectin. (Akchurin & Kaskel, 2015)

The NHANES results showed that there is an association between CKD and higher levels of leptin and adiponectin. Akchurin and Kaskel (2015) stated that pathophysiology inflammation may not be the same in CKD patients of various racial and genetic backgrounds. They found that Caucasians have a stronger affiliation of adiposity and markers of inflammation when compared against African Americans.

According to Ramos *et al* (2008) recent studies have indicated that CKD is associated with both inflammation and oxidative stress. These factors may also largely contribute to the excessive cardiovascular disease as seen in CKD patients. It was also found that Body Mass Index (BMI) and body fat percentage were significantly linked with the measured markers of inflammation and oxidative stress within the CKD study group (Ramos *et al*, 2008).

Mallamaci and Tripepi (2013) stated that there is a significant decline in renal plasma flow as body mass increases, excluding obesity with BMIs of  $> 30 \text{ kg/m}^2$ .

Briffa *et al* (2013) concluded that when a person is suffering from obesity, there are two main cellular points that are linked to proteinuric renal disease:

- i. Structural changes to the glomerulus, therefore allowing more proteins to enter the urine.
- ii. The proximal tubules are unable to reabsorb the increased protein load.

Inflammation is generally termed as a response of an organism to any injury; referring to any process causing cellular or tissue damage (Vianna *et al*, 2011). Exacerbated tissue damage results in activation of the immune system thus enhancing the inflammatory response which facilitates disease onset and progression. For example, CKD with obesity or hypertension as the cause of inflammation. Vianna *et al* (2011) stated that inflammation is being more and more recognised as role-player in the pathophysiological process of loss of nephron function.

### 1.2.3.2 Other

#### i) Communicable diseases: HIV and HIVAN

HIV is an epidemic in SSA and through the screening of the population, it was found that in 5-83 % of HIV-infected patients, in this region, showed kidney involvement (Webster *et al*, 2016), however Mallipattu *et al* (2014) found there is a lack of information regarding the SSA population which is troubling as SSA accounts for two-thirds of the global HIV population.

HIV-associated nephropathy (HIVAN) is recognised as a separate condition from HIV itself, and in the 1980's HIVAN was found to be the leading cause of CKD and ESRD in HIV infected patients. (Ross and Klotman, 2004)

HIVAN sufferers display proteinuria within nephrotic range, however they lack signs of peripheral oedema. HIVAN patients commonly exhibit no hypertension, which is a unique trait in renal disease and HIVAN. Another finding is that HIVAN patients are most often HIV-seropositive patients who experience a decrease in their ability to store sodium. This

coupled with the above HIVAN characteristics suggest that HIVAN is a salt-wasting disease. (Ross and Klotman, 2004)

Ross and Klotman (2004) stated that the only proven reliable test to establish or eliminate the presence of HIVAN is a renal biopsy. The presence HIVAN will manifest in the glomerular, tubular, and interstitial compartments of the kidney.

In the study conducted by Ross and Klotman (2004), HIVAN was most commonly associated with black ethnicity. This suggests that genetic factors are involved. Mallipattu *et al* (2014) stated that HIVAN is seen most in the genetically susceptible individuals from West Africa and not as prominent in the Caucasian population. Jha *et al* (2013) speculated that this is related to the differential prevalence of high-risk alleles in *MYH9* and *APOL1*.

Early treatment with anti-retroviral (ARV) drugs has been observed to reduce the onset of HIV-associated nephrology, however such early use of ARV drugs has been found to carry the risk of nephrotoxic effects, thus contradicting the beneficial properties of ARVs. These nephrotoxic effects include: electrolyte disorders, tubular toxic effects, interstitial nephritis, lactic acidosis, and crystal-induced obstruction (Jha *et al*, 2013). There is an increasing trend of CKD associated ARV nephrology with increasing exposure to atazanavir and indinavir (Mocroft *et al*, 2010). The same study also stated that the use of tenofir within a 12 month period resulted in a 4-fold increase in CKD incidence amongst Aids patients, compared to those who had never been treated with tenofir. In contrast to the above information, Szczech *et al* (2002) found that ARV treatment using protease inhibitor treatment resulted in a decrease in the decline of creatinine clearance in clinically diagnosed HIVAN patients. These conflicting finding make the benefits of ARV treatment for kidney function ambiguous.

A different treatment option is angiotensin converting enzyme (ACE) inhibitors. Such drugs have been demonstrated to prevent or slow the development of renal failure (Ross and Klotman, 2004). These finding were supported by a study conducted by Kimmel *et al* (1996).

Prednisone was also studied as a possible treatment to slow renal failure development in HIVAN patients. It was found that creatinine clearance improved while on prednisone, however when patients were tapered off treatment they experienced renal relapse (Ross and Klotman, 2004). Szczech *et al* (2002) conducted a retrospective study on 19 patients suspected to have HIVAN. The 5 patients that were treated with prednisone experienced a clear increase in creatinine clearance by 5.57 mL/min/ month, while the 14 untreated patients experienced a creatinine clearance decrease of 3.32 mL/min/month. Therefore prednisone is a good treatment for maintaining and improving kidney function, however it needs to be a lifelong treatment as tapering off leads to renal relapse.

Without any of the above treatments, most patients suffering from HIVAN will progress to ESRD within 1-4 months of diagnosis, therefore making the mortality of HIVAN a rather alarming statistic. (Ross and Klotman, 2004)

Other specific communicable infections such as the hepatitis B and C viruses, worldwide, cause severe kidney lesions leading to nephrology.

## **ii) Traditional Medicine:**

The use of certain herbs in traditional medicine, namely in Africa and Asia, has been found to have nephrotoxic effects (Luyckx *et al*, 2005). This occurs when potentially toxic herbs are ingested, most commonly through the incorrect substitution of harmless herbs with toxic

herbs. Sometimes contamination of harmless herbs occur involving heavy metals or interactions between herbal medicine and conventional medicines. Renal injury has been reported with association to several herbs. The most common herb associated with CKD is aristolochic acid (AA). Ingestion of this herb is associated with progressive interstitial nephritis (Jha, 2010).

### iii) Water:

These include various disorders that directly or indirectly related to water. Water scarcity, especially in tropical regions, leads to the risk of dehydration resulting in the accumulation of waste products in the blood due to reduced kidney function (Rango *et al*, 2015).

Contaminated water may also be harmful to the kidneys during filtration. Harmful contaminants include heavy metals and organic compound that can be leached from soil (Soderland *et al*, 2010). Heavy metals include lead, cadmium, arsenic, mercury, and uranium. Such heavy metals often lead to exposure through the workplace (Soderland *et al*, 2010). Waterlogged fields and stagnant water often become contaminated with waterborne diseases, such as malaria and *Escherichia coli*, and can damage the kidneys (Jha *et al*, 2013).

## 1.2.4 Identifying CKD

The identification and stage classification is based on the measurement of glomerular filtration rate and albuminuria. However calculating the actual glomerular filtration rate by the measurement of urine exogenous substances is tedious and impractical. Therefore the eGFR is used, as previously mentioned and illustrated in Table 1.1. This method of measurement is based on the serum creatinine concentration.

Shimada *et al* (2016) explains that creatinine is a waste product derived from the breakdown of creatine phosphate in the body's muscle metabolism. Approximately 2 % of the body's creatine content is metabolised into creatinine each day. This results in a daily creatinine production occurring at a relatively steady rate.

The creatinine is therefore a waste product that is freely filtered through the glomeruli of the kidneys. The creatinine is also secreted by the proximal tubules of the kidney, however this is in very small amounts. (Shimada *et al*, 2016)

According to Mohabbati-Kalejahi *et al* (2012) the first creatinine measurement method was developed in 1886 by Max Jaffe and was thus name the Jaffe reaction. This method involves a chemical reaction between creatinine and picrate in an alkaline solution. This reaction produces an orange-red complex that is measured spectrophotometrically and is prone to interferences from certain blood constituents such as ketones, bilirubin, certain antibiotics and pyruvate to name a few. This reaction is also sensitive to temperature and pH.

Due to these interferences, there was a need to develop alternate methods of creatinine measurement. The enzymatic creatinine assays, with less interference were developed, utilizing enzymatic reactions involving either creatininase or creatinine deaminase. (Mohabbati-Kalejahi *et al*, 2012)

The last and most accurate method of creatinine measurement, mentioned by Mohabbati-Kalejahi *et al* (2012), is the isotope dilution mass spectrometry. This method is considered to be the Gold Standard of creatinine measurement due to it having a very high specificity and accuracy. However this method is costly and has a low throughput therefore it is not used as a routine form of testing in most laboratories.

As previously mentioned eGFR is a calculated value that is used when testing and classifying renal function or disease. This method of measurement is quick and practical when applied to settings such as local clinics where many patients are attended to daily. eGFR is an estimation equation, used to calculate



the GRF incorporating the result from a serum creatinine test, gender, age, body size, and race. This quick form of measurement is useful in the staging of kidney disease, such as CKD, as well as in formulating a treatment plan based on the severity of decreased eGFR. (NKF: Creatinine: What is it? – <https://www.kidney.org/atoz/content/what-creatinine>)

Several estimation equations exist. The equations mostly used are the “Modification of Diet in Renal Disease” (MDRD) equation and the “Chronic Kidney Disease Epidemiology Collaboration” (CKD-EPI) creatinine equation.

Existing equations need to be reviewed for accurate assessment of the differences involved by ethnic origin, region, or possibly both in order to validate said equations for obtained eGFR values, as per ethnicity, against the reference method eGFR values. Until this has been achieved, it is advised that the modified MDRD or CKD-EPI equation be used with acknowledgment of the possible occurrence of variation in results thus leading to misclassification of CKD. It is for this reason that POCT creatinine devices based on eGFR measurement are used as screening tools and not diagnostic tools. (Jha et al, 2013)

### 1.2.5 Creatinine levels as a measure of CKD

Serum creatinine remain the most common biochemical parameter to determine the eGFR and assess kidney function. Creatinine is formed in the muscle at a relatively constant rate through the dehydration of creatine. The conversion is irreversible therefore making it a viable marker to measure. The amount of creatinine formed per day is directly related to one’s muscle mass, which of course varies between gender, age, and ethnicity. (NKF: Creatinine: What is it?)

The creatinine compound is freely filtered through the kidneys’ glomeruli, without being appreciably reabsorbed or secreted by the kidney tubules. This results in the serum creatinine concentration forming a balance between its production and the GFR. Therefore, if the GFR decreases, while creatinine formation remains constant, the serum creatinine levels will rise. Creatinine levels are used to calculate the eGFR, and even though the eGFR has several limitations, it is able to detect reduced GFR at relatively early stages of kidney disease. This makes it an extremely useful tool in CKD screening.

### 1.2.6 Estimated Glomerular Filtration Rate

The GFR refers to the measurement of how well the kidneys are filtering waste products from the blood stream. GFR has been found to be the best overall measure of kidney function. The gold standard for measuring GFR involves administering inulin, a group of naturally occurring polysaccharides, and subsequently measuring its clearance. This is however not routinely done as it tedious and expensive. Therefore specialized equations have been developed, namely the MDRD equation which takes into account age, gender, and ethnicity, in order to obtain an eGFR value. (Botev *et al*, 2009)

#### i. MDRD and Cockcroft-Gault equations:

eGFR is determined through either of the following two equations: the Modification of Diet in Renal Disease (MDRD) equation or the Cockcroft-Gault (CG) equation. A review study conducted by Zhang *et al* (2008) found that the MDRD equation was reported as more accurate in determining CKD status in elderly patients, 65 years and above, whereas the CG equation overestimated eGFR values.

Levey *et al* (2005) reported that the MDRD equation performed substantially better in estimation of eGFR in people with diagnosed CKD and with people who had an eGFR of

<90 mL/min/1.73m<sup>2</sup> compared to that of the CG equation. An additional draw-back of the CG equation is that it does not consider ethnicity. This makes the MDRD equation the most clinically favoured equation when estimating eGFR. Levey *et al* (2005) suggest that the additional use of novel, emerging biomarkers such as cystatin C are important factors to study in order to improve upon the estimation of eGFR across genders, ages, and ethnicity.

The MDRD equation was introduced in 1999 (Ali *et al*, 2013). Rostoker *et al* (2009) stated that the MDRD equation was first developed in a population of patients with severe renal function impairment. The CG equation was previously introduced in 1976 (Rostoker *et al*, 2009) and was previously developed in a group of 236 Canadians, mostly male (Noble & Johnson, 2007).

Inulin and isotope measurement methods are used as the gold standard method in validating the accuracy of eGFR determination by MDRD and CG equations (Ali *et al*, 2013). The MDRD equation predicts eGFR and is as follows:

$$eGFR = 186 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times 0.742 \text{ (if female)} \times 1.212 \text{ (if black)}$$

(Munikrishnappa, 2009)

Munikrishnappa (2009) stated that the MDRD equation has been found to be less accurate at eGFR levels above 60 mL/min/1.73m<sup>2</sup> compared to the CG equation. This finding was also supported by Ali *et al* (2013). This equation also performs significantly poorer in subjects without renal disease (Lin *et al*, 2003). Lin *et al* (2003) found that the MDRD equation exhibited less bias than the CG equation, and was also more precise in general. However the MDRD equation gave results with a smaller median absolute error compared to the CG equation, as stated by Rostoker *et al* (2009). Lin *et al* (2003) stated that the MDRD equation has been extensively evaluated in both Caucasian and African American populations and makes adjustments for these two ethnic groups.

The CG equation predicts creatinine clearance (CrCl) in place of eGFR and is as follows:

$$CrCl \text{ (mL/min)} = \frac{[(140 - \text{Age in years}) \times \text{Weight in kg}]}{Scr \text{ (mg/dL)}} \times 72 \times 0.85 \text{ (if female)}$$

(Munikrishnappa, 2009)

CrCl generally overestimates eGFR thus making the CG equation a slight overestimation itself (Muniskrishnappa 2009). However, studies have shown that the CG equation underestimates eGFR in elderly patients, especially at higher eGFR values (Lee *et al*, 2009). eGFR values calculated by the MDRD and CG equations were the same in subjects with an eGFR < 30 mL/min/1.73m<sup>2</sup>, but the CG eGFR was higher than that of the MDRD eGFR in subjects younger than 50 years of age and lower in subjects older than 60 years of age (Lee *et al*, 2009). These differences increased with age.

Differences between the two equations are as follows; the CG equation can be corrected for using body surface areas while the MDRD equation corrects for race. The CG equation measures CrCl while the MDRD equations measures eGFR directly.

Similarities between the two equations include; both equations take into account gender. Both equations can be used interchangeably at CKD stages 1 and 4 (Ali *et al*, 2013)

One of the several limitations of the eGFR is that it is not suited to all clinical situations, for example: it is not ideal for paediatric (<18 years) and elderly patients, pregnant

women, patients with skeletal muscle disease, and patients with rapidly fluctuating kidney function. (Kidney Health Australia: Chronic Kidney Disease (CKD) and eGFR)

The Modification of Diet in Renal Disease (MDRD) equation is the most widespread used equation for calculating eGFR. This equation was designed to register a decrease in GFR, however it does not work well when it comes to values above 60 mL/min/1.73m<sup>2</sup>. Therefore results above this value are reported as >60 mL/min/1.73m<sup>2</sup>. A more accurate and concise test for detecting reduced GFR involves a 24 hour urine test in which 24 hour creatinine clearance is monitored where both the urine and serum creatinine levels are measured. This test is uncomfortable and tedious and therefore is mostly used in drug dosing (Horowitz et al, 2014).

**Table 1.2:** Limitations and advantages of the MDRD and CG equations (adapted from Munikrishnappa, 2009).

| Equation         | Limitation   | Advantage   |
|------------------|--|---|
| Both MDRD and CG | <ul style="list-style-type: none"> <li>• Renal and extra-renal conditions affecting steady state of creatinine in plasma</li> <li>• Interference with creatinine assay</li> <li>• Variation in muscle mass and diet</li> </ul>   | <ul style="list-style-type: none"> <li>• Take into account gender</li> </ul>  |
| MDRD             | <ul style="list-style-type: none"> <li>• Reliability and accuracy decreased in eGFR extremes</li> <li>• Lack of validation in some ethnic groups</li> <li>• Equation not adapted for drug dosing for renal impairment patients</li> <li>• Requires a computer for data processing</li> </ul> | <ul style="list-style-type: none"> <li>• Takes into account race ethnicity</li> </ul>   |
| CG               | <ul style="list-style-type: none"> <li>• Calibration bias</li> <li>• Values that are not adjusted for body surface area</li> </ul>   | <ul style="list-style-type: none"> <li>• Improved accuracy in elderly patients</li> <li>• No computer required, results can be calculated at bedside</li> </ul> |

**ii. Ethnicity and eGFR:**

According to Xue *et al* (2007), people of African American descent and several other minority ethnic groups are more prone to developing end-stage renal disease (ESRD) and CKD compared to that of Caucasian people. However research is only able to partially explain this phenomenon. The study found that the other minority groups were prone to CKD and ESRD even when analyses were adjusted for age and gender. This result was also found regardless of the participants' diabetes and hypertension statuses. This finding suggests that diabetes and hypertension are not a factoring influencing ethnic differences in development of CKD and ESRD when compared to Caucasians.

The study also found that socioeconomic status, access to health care, and co-morbid conditions did not contribute to the minorities developing CKD and ESRD. This finding was stated to be supported by several other studies, including those conducted by Hall *et al* (2004) and Hsu *et al* (2003).

Interestingly enough, the study by Xue *et al* (2007) found that although the incidence of ESRD and CKD was higher in the Black population, their mortality rate was lower than that of the Caucasian population. It was suggested that this could be explained by Black patients starting RRT earlier than that of White patients.

This study also discussed that a possible reason for the higher incidence of CKD and ESRD within the Black population is due to CKD being diagnosed based on eGFR values obtained through the measurement of creatinine levels. This is an important factor in racial differences in CKD incidences because it has been found, and supported by other studies (Obrador *et al*, 1999), that physiologically the Black population have naturally higher serum creatinine levels than that of Caucasian people. Although eGFR is not the sole criterion for diagnosing CKD, it is a very important factor. Therefore this could possibly explain the racial differences in CKD and ESRD incidence rates.

With regard to the above study, one can assume that physiological differences between races should be further studied in order to ascertain what other possible differences, apart for serum creatinine levels, could be contributing to this difference in incidence rates.

A study performed in a black South African population has concluded that the 4-variable MDRD equation can be used without the ethnicity correction factor of 1.212. This however cannot be generalised to all black population within South Africa because of racial diversity within the South African black population (Van Deventer H, *et al*. 2008).

#### 1.2.6.1 Standardization of eGFR equations

According to Earley *et al* (2012) there are 5 criteria that need to be met when developing, validating, and standardizing GFR estimation equations:

- 1.2.6.1.1 The equation should be developed and validated in separate populations, and both populations should represent the intended population. There should be a large enough sample size in order to detect a significant improvement in the equation's performance.
- 1.2.6.1.2 The measured GFR should be based on either urine or plasma clearance of an exogenous filtration marker.
- 1.2.6.1.3 The serum creatinine assay used in the development and validation data sets should be standardized against standard reference material, such as IDMS traceability.
- 1.2.6.1.4 Measurements of the equation should include precision, bias, and accuracy.
- 1.2.6.1.5 Validation must include the comparison of another newly developed equation with more than, or equal to one previously developed equation within the population of interest in order to determine which equation is superior. A good comparison to use is the unmodified CKD-EPI equation (Earley *et al*, 2012)

#### 1.2.7 Albuminuria

Albumin is a fairly large protein produced by the liver and which is used to regulate colloid osmotic pressure (COP) within the body (Nicholson *et al*, 2000). Albuminuria (previously known as microalbuminuria) is a condition in which small amounts of albumin have entered the urine. When proteins are lost through urine, it results in a decrease in COP and thus results in an increase of filtration across the capillaries. This leads to an accumulation of fluid within the bodily tissues, which can lead to pulmonary oedema (Nicholson *et al*, 2000).

Measurement of albuminuria is an easy method to test for kidney damage. This is because larger molecules such as proteins are important for the body and thus do not filter through the kidneys for excretion. The reason for albumin being an easy marker for kidney damage is because it is one of the first proteins to enter the urine when there is kidney damage. (NKF KDOQI Guidelines: [https://www2.kidney.org/professionals/KDOQI/guideline\\_diabetes/guide1.html](https://www2.kidney.org/professionals/KDOQI/guideline_diabetes/guide1.html))

The American Diabetes Association (ADA) states that the gold standard test for measuring urine albumin clearance is a 24 hour urine measurement, however this method of measurement is tedious and time consuming. It is also open to possible errors in collection methods and sample storage. A newer and more convenient method of measurement is the spot urine albumin/creatinine ratio (ACR). This spot urine must be collected under standardized conditions, which are stipulated to be the first voided and midstream urine specimen, in order to detect microalbuminuria. The ADA and National Kidney Foundation (NKF) define albuminuria when a patient has an ACR between 30 and 300 µg/mg for both male and female. However this definition does not take into account gender differences in creatinine excretion (Mattix *et al*, 2002).

Derhaschnig *et al* (2002) stated that it is unclear if the measurement of albuminuria alone is enough to advocate kidney damage, or if it is necessary to combine this measurement with an ACR test. Both methods of measurement can be conducted on the same spot urine sample, making it convenient to conduct both tests instead of just one of the two. It would be preferable to conduct both tests when testing for CKD because the ACR test includes the creatinine component of kidney filtration which is the main marker in determining CKD status.

Through the results obtained from creatinine eGFR measurement, albuminuria measurement, blood pressure, and blood sugar measurements medical practitioners are able to make a reliable and accurate diagnosis of a patient's kidney health status.

### **1.2.8 Treatment for CKD: Prevention and Screening**

The NKF recognised the important role which family physicians play in detecting and managing CKD in patients, especially with patients in early stages of CKD where medical intervention is most effective. Therefore the NKF set out to develop guidelines for physicians to follow in order to manage CKD patients. (NKF Fast Facts Sheet: <https://www.kidney.org/news/newsroom/factsheets/FastFacts>)

CKD often goes undetected until stage 4 is reached. This is due to the fact that stages 1-3 progression is slow and asymptomatic. This creates a need for patients deemed "at increased risk" to be screened and monitored for CKD development and/or progression. Patients deemed to be "at increased risk" are those who already suffer from diabetes and/or hypertension. Patients suffering from afore mentioned conditions can be managed by controlling and treating their pre-existing conditions, the onset of CKD could be slowed or even possibly prevented. (Johnson *et al*, 2004)

When a patient, without any pre-existing conditions, is diagnosed with CKD it is advisable to start aggressive treatment against hypertension and cardiovascular disease. This is because these conditions have been found to develop during CKD progression and also increase the rate of CKD progression. Preventing these complications from arising can result in a decreased mortality rate.

CKD treatment can also involve changing the patient's diet, lifestyle, and medication. A new diet should be followed which consists of reducing the intake of sugar, sodium (which will help decrease blood pressure), cholesterol (which will prevent heart and blood vessel disease), and control the intake of carbohydrates which are converted into sugars upon metabolism. A well-studied and popular diet is a protein restricted diet that still provides adequate amounts of amino acids and energy (Bellizi *et al*, 2016).

Lifestyle changes should involve weight management, limited alcohol intake, regular exercise, and patients who smoke must quit smoking. Medications to aid in managing CKD include angiotensin converting enzyme inhibitors or angiotensin receptor blockers, both of which protect the kidney and lower the risk of cardiovascular disease and diabetes mellitus. (NKF Fact Sheet: [www.kidney.org](http://www.kidney.org)).

Once a patient has reached stage 4 of CKD, treatment involves controlling pre-existing and resultant conditions as well as preparation for renal replacement therapy, being either dialysis or kidney



transplantation. (Johnson et al, 2004). Any medication taken by the patient should have its dose re-evaluated and altered due to decreased kidney function.

By the time a patient has reached stage 5 of CKD, they have end-stage renal failure. The only treatment is to continue renal replacement therapy. Renal failure cannot be cured and thus the best treatment for it is prevention (Johnson et al, 2004).

### **i) Prevention**

There are many treatment plans that can slow the progression of CKD as well as reducing the risk of CVD mortality. The most common treatment plans involve the management of blood pressure, by use of medication that inhibits the renin-angiotensin pathway, glycaemic control through diet modification, and lipid-lowering therapy, regardless of the patients starting concentration of cholesterol. (Jha et al, 2013)

Lipid-lowering therapy reduces the chances of an atherosclerotic event occurring in patients suffering from CKD. Prevention of further deterioration of impaired kidney function is also very important. This will include optimizing the salt and protein intake of a patient with CKD. This will aid in controlling blood pressure and prevent excess proteinuria (Jha et al, 2013).

The last effective measure of preventative treatment is self-management, which involves improving one's lifestyle, diet, adherence to treatment, as well as their knowledge of their disease. Self-management is a cost-effective manner of treatment for patients suffering from stage 3 CKD. According to Jha et al (2013) this is being investigated in a randomised trial.

### **ii) Screening**

Cost-effectiveness of screening the general population for CKD is still unclear. Screening for CKD using eGFR to identify patients who will benefit most is poorly characterized and investigated. Most population-based screening approaches have been implemented in developed countries, however due to the population variations of risk factors, one cannot screen all ethnic populations based on the same risk factors as this will lead to the possibility of some groups' CKD status being misclassified. However population based screening for the relevant risk factors, such as HIV, diabetes, hypertension, and CVD in SSA, may have the advantage of increasing health awareness in developing countries and aid in educating the population with regard to the risks involved in developing such diseases. If the population is more aware of the risks that such diseases pose to their health and well-being, it may lead to the public taking an interest in their diet and lifestyle and thus may reduce the disease burden on the country's health care system and budget. (Jha et al, 2013)

## **1.3 POINT-OF-CARE TESTING**

### **1.3.1 Introduction**

Point of Care Testing (POCT) is defined as an analytical test that is performed near the patient, delivering results in real time without the need for a conventional laboratory. Such tests can be carried out at the patient's bedside in hospital or in the consultation room of a local clinic. Other possible locations include ambulances, and all sectors of a hospital (ICU, theatre, surgical wards, and consultation rooms). (Tirimacco, 2013)

POCT instruments can be handheld devices, desktop analysers, or even non-instrumental which would make use of, for example, reagent strips instead of a machine. (Tirimacco, 2013)

POCT is a promising form of medical screening which allows for large populations of people to be screened for possible diseases, such as diabetes mellitus. It eliminates the waiting period for the return of conventional laboratory results, which in turn may lead to increased clinical performance with regard to the number of patients attended to per day. POCT also allows for smaller less invasive sample collection, however these benefits can only be optimally achieved if cooperation with the central laboratory is achieved. (POCT Implementation Guide: 2008)

Some POC instruments can be used by patients in the comfort of their own home, such as a glucose meter, thus resulting in patients taking a greater responsibility for their own health.

There are however several disadvantages which include; the possibility of inaccurate use of the instrument resulting in inaccurate results. This could lead to the need for additional testing, and thus increased consultation time and waiting period. Poor record keeping of the quality controls (QC) and the quality of results is also a concern. As well as the linearity of the POCT results compared with those of a conventional laboratory. (POCT Implementation Guide: 2008)

If POCT instruments could be evaluated and validated, proving that they are as accurate in their results as those obtained from conventional laboratories, then POCT has great potential in changing the conventional patient diagnosis procedure for diseases such as diabetes mellitus, chronic kidney disease, and cardiovascular disease.

POCT can also be the solution to many health care issues in developing countries. This can be seen in the significant reduction in unnecessary anti-malaria treatment during the last decade, through the use of POCT. This highlights the transforming potential of POCT for futures to come. However there will always be a need for central laboratories as they offer qualified clinicians and physicians who can provide consultation and guidance when it comes to result interpretation. These laboratory personnel can identify any pre-analytical and post-analytical errors, recommend follow up tests if POCT yields worrisome results, and also act as POCT coordinators of quality management.

POCT has also been defined as a “disruptive innovation” (Luppa et al, 2016) that is a sustaining technology, with the potential to provide healthcare with innovative performance metrics.

Limitations for further evolution of POCT come in several forms, namely: the establishment of new and reliable parameters, finances where institutions cannot financially keep up with the technological equipment advancements, as well as institutions being unable to keep up with the evolving changes in clinical diagnostic methods due to technological advances, and finally: POCT evolution and advancement is often hindered through the market failure of non-invasive devices which have been in the developmental pipeline for several decades before being cancelled (Luppa et al, 2016).

### **1.3.2 Methods of sampling and sample sources** (Luppa et al, 2016)

- Invasive: whole blood samples (venous/arterial)
- Moderately invasive: capillary blood
- Non-invasive, but sampled: urine, saliva, tears, sweat, faeces, sputum, etc.
- Non-invasive and not sampled: direct detection methods applied to the skin surface/plane (electroporation, optical techniques, temperature measurement).

### **1.3.3 Diabetes in Sub-Saharan Africa and the importance of POCT assessment**

SSA is familiar with wars, malnutrition, and communicable diseases; subsequently focus has been on emergency response services, food care packages, as well as communicable disease prevention. This has left non-communicable diseases unaddressed in most parts of SSA. This is a common occurrence even though the non-communicable diseases are responsible for a large share of disease burden and have been dubbed as “The Silent Killer” (Renzaho, 2015). Diabetes mellitus is the biggest silent killer and



was previously considered to be rare in rural African regions. This has changed over the last several decades and diabetes has been found to be a severe burden in SSA.

The rising incidences of diabetes in SSA regions are most prominent in urbanized areas. Mbanya *et al* (2010) stated that the estimated growth and urbanization of SSA will increase from 12.1 million in 2010 to 23.9 million by 2030. This shift of urbanization has resulted in the SSA traditional diet, which is high in fibre, low in fat and salt, to be replaced by a more westernized diet. Westernized diets are energy dense, highly refined, and have a high salt and fat content. The urbanized life style is also more sedentary and thus results in a decrease in physical activity (Renzaho, 2015). The above can account for higher diabetes incidence rates within urban regions compared to those of rural regions. Risk factors associated with the westernized diet and a more sedentary lifestyle include increased body-mass index, waist circumference, waist-to-hip ratio, and total body obesity. It is challenging to get high risk patients to lose weight and improve their eating habits as it is a common cultural belief that obesity is a sign of affluence. This belief has been instilled through the persisting poverty and destitution within SSA, thus making inhabitants reluctant to shed excess weight. (Mbanya *et al*, 2010)

In several SSA screening studies it was found that less than 50% of the participants were aware of their diabetic status, where as in South Africa more than 50% of the participants knew what their status was. This could be explained by South Africa having better and more easily accessible health care facilities, compared to those in SSA (Mbanya *et al*, 2010). In SSA, serious challenges faced by diabetic patients include the lack of constant access to antidiabetic drugs, namely insulin. In addition to this, such drugs are not easily affordable. This makes diabetes management almost impossible and thus the resulting incidence rates lead to an increase in morbidity and pre-mature mortality. This in turn reflects heavily on the government and health care system in the form of additional socioeconomic costs.

Mbanya *et al* (2010) stated that the prevalence of diabetes increases with age, starting from the age of 55 years old (Mortala *et al*, 2008). It was also stated that sex was not a contributing factor as it had little effect on diabetes in SSA. Another contributing factor to the increasing prevalence of diabetes in SSA is the chronic burden of communicable diseases, namely tuberculosis (TB) and HIV. It has been found that patients receiving anti-retro viral (ARV) medicines have an increased prevalence of diabetes. This could be explained through the metabolic pathways that are affected by highly active anti-retroviral therapy (HAART), which include the direct effects of HAART, the pro-inflammatory process of HIV, and also the indirect effects of HAART which includes a change in body fat distribution. TB results in an increased risk of diabetes due to an increased risk of pancreatitis and insulin deficiency. TB also complicates diabetes management (Renzaho, 2015).

Most SSA countries' abilities to respond effectively to the diabetes epidemic is greatly hindered by the lack of funding. If the countries could implement mass screening programs, then diabetic sufferers could be identified earlier and thus managed easier. The screening programs would also be able to identify those at high risk and thus allow for early implementation of measures to prevent the progression of the disease. POCT would be an ideal form of mass screening as the devices are small, robust, and have quick analysis time. If such devices could be placed in local clinics then more people could be screened instead of just those visiting the far and few between hospitals.

Another interesting finding by Renzaho (2015) was that most SSA inhabitants rely on traditional healers for medical attention. This could be useful as most traditional healers have embraced biomedical technologies and thus would possibly consider being trained in POCT. This would open up diabetes screening to a much larger population. If found to have diabetes or be at risk then the traditional healer could refer the patient to medical practitioner for further treatment and management.

If none of the above issues are addressed, SSA may be facing a new epidemic as large, if not larger, than the HIV epidemic.

### 1.3.4 Current POCT Analytical Devices (Luppa et al, 2016)

Currently there are several popular methods of POCT available today such as complex biosensor-based devices, test strips, lateral-flow tests, benchtop POCT analysers, and Lab-on-chip (LOC) devices.

#### **The Novastatsensor® makes use of the complex biosensor-based method:**

Miniaturised biosensor systems have been developed in the last two decades, and can detect analytes by applying two forms of detector components: biological and physiochemical. Biological fluids such as whole blood, saliva, serum, plasma, urine etc. contain components that interact with the biological components on the recognition layer, which is fixed on the solid-state surface of the sensor inside of the device. At the transducer surface, biospecific reactions are read out through the use of either optical, microgravitational, or electrochemical methods.

### 1.3.5 Current POCT Analytical Techniques (Luppa et al, 2016)

Through the miniaturisation of electronics and optics, portable devices have been developed which, when having completed stringent validation studies, can produce results that are aligned with results obtained from main central clinical laboratories. These miniaturized analytical techniques are based on mostly electrochemical methods, optical transduction, and chromatography. Currently the electrochemical and optical techniques are most favoured, these include: electrochemical based devices, optical based devices, and mass-sensitive based devices.

### 1.3.6 Emerging next-generation POCT

POCT is estimated to rise in countries with large populations, the latter resulting in an increase in the prevalence of infectious and chronic diseases. This leads to a need for POCT devices that are affordable, robust, and unrefrigerated in order to provide optimal sensitivity and specificity, while involving minimal steps and rapid turn-around time (Vashist *et al.* 2015). The development of these technologies would be well suited for rural regions such as those found in SSA where storage facilities and medical funding are limited and patients do not have the means to make multiple trips to clinics, therefore making this form of up and coming technology relevant to all POCT research and development.

#### **1.3.6.1 Strategies for Prolonged Reagent Storage and Shelf Life**

The most common strategies entail the use of sugars, sugar alcohols or stabilizers, reagent pouches, freeze drying, or encapsulation of biomolecules within natural polysaccharides. One example is a non-ionic natural polysaccharide, called pullulan, which forms an oxygen-impermeable solid when dried. This allows for storage of unstable biomolecules as stable water-soluble pellets, lasting several months at room temperature. This is particularly useful in developing countries' rural regions where adequate electricity and refrigeration is lacking. (Vashist *et al.* 2015)

#### **1.3.6.2 Challenges**

There are still many challenges facing the development of POCT devices. The number one concern and challenge is device demonstration of high analytical precision and reproducibility for analyte quantification when large numbers of patient samples are involved. This needs to be done with minimal or zero interference by potential nonspecific substances. (Vashist *et al.* 2015)

The integration of microfluidic systems with POCT has proved to be challenging due to the difficulties faced in the miniaturization of components that are required for the active transport of liquids. Additionally there is the challenge of reagent long-term storage and usage. (Warade, 2014)

In the case of the previously discussed CP-based POCT, there is the concern with regard to hygiene and proper disposal of contaminated objects, such as test strips or cartridges. These issues need to be resolved before CP-based POCT devices can be released to the public for private self-monitoring (Warade, 2014).

Due to the dynamic and ever increasing use of the internet and electronic communication devices, there is major cause for concern when it comes to patient record confidentiality, security, and ownership. Patient information needs to be secured behind advanced encryptions and algorithms which can prove costly to develop and implement. In addition to this problem, there is the obstacle of creating user platforms and interfaces in order to allow for patients to access their data. As well as a bidirectional communication between the POCT devices and said user platforms and interfaces. Currently password and usernames are implemented as a form of ensured privacy and security. (Vashist *et al.* 2015)

The last and equally important challenge to overcome is the economic feasibility, which is a major determinant when it comes to commercialization of POCT devices. Complex instrumentation and reagent or cartridges result in the need for extensive, and often costly, training of health care personnel. Test strips, such as urine dip sticks, are a good example of economic feasibility as they are cheaper than most other POCT devices, they also require only basic training and are extremely simple to use. This highlights the need for the development of cheap and user friendly methods for POCT. (Warade, 2014)

An often overlooked factor to consider is the involvement of private medical insurance companies as the cost implications of POCT may affect patients' reimbursement policy and monthly premiums. This leads to the dire need for the involvement of the industry sector in the early critical stages of POCT device commercial development. (Vashist *et al.* 2015)

### **1.3.7 POCT Operator Training and POCT Management**

Often the POCT operator has no background training in laboratory medicine, even when used in professional medical environments. For example: patients conducting self-monitoring from home, or nurses in hospitals and clinics. This largely results in errors occurring in POCT due to operator error instead of device related failure. The most commonly found errors include: operators failing to carry out basic (1) device preparation, (2) maintenance and QC steps, (3) non-adherence to standard test procedures, and (4) the use of expired reagents. This emphasises the need for the implementation of numerous guidelines and policies that call for the formal training of POCT operators in order to reduce these avoidable errors. (Luppa *et al.* 2016)

The International Standards Organisation (ISO) established the compulsory guidelines for ensuring a universal quality control standards and competence worldwide.

ISO 22870:2006 was implemented based on ISO 15189 and has been updated to ISO 22870:2016 ((ISO website: [http://www.iso.org/iso/home/store/catalogue\\_tc/catalogue\\_detail.htm?csnumber=71119](http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=71119)), with special emphasis placed on POCT.

The principle of ISO 22870:2016 puts emphasis on operators' qualifications using requirements for quality and competency (ISO website: <https://www.iso.org/obp/ui/#iso:std:iso:22870:ed-2:v1:en>). It also emphasises the appointment of a manager for the practical and theoretical training of POCT operators before being declared qualified for POCT (Luppa *et al.* 2016). It includes the appointment of a quality manager who is responsible for the design, implementation, and operation of quality control that will ensure the POCT conforms to the quality standards of the central laboratory. ISO 22870:2006 also places emphasis on Internal Quality Control (IQC) and External Quality Control (EQC). These guideline implementations are applicable to both hospital environments and outpatient clinics, only excluding home care usage.

| Device               | Statsensor                   | i-STAT                       | ABL 800 Flex                 | Reflotron                      | Dri-Chem 400                  | Piccolo                             |
|----------------------|------------------------------|------------------------------|------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Manufacturer         | Nova Biomedical              | Abbott POC                   | Radiometer                   | Roche                          | Fuji-Film                     | Abaxis                              |
| Method principle     | Enzymatic with amp biosensor | Enzymatic with amp biosensor | Enzymatic with amp biosensor | Enzymatic with dye refelctance | Enzymatic with dye absorbance | Enzymatic with indicator absorbance |
| Consumable           | Dry strip                    | Cartridge                    | Sensor cassette              | Dry strip                      | Dry slide                     | Rotor                               |
| Sample type          | Whole blood A/C/V            | Whole blood A/C              | Whole blood A/C/V            | Whole blood V/C                | Serum or plasma               | Whole blood, serum, or plasma       |
| Sample volume (µl)   | 1                            | 65                           | 125 C 250 Syr                | 30                             | 10                            | 9                                   |
| Analysis time (min)  | 0.5                          | 2                            | 1                            | 2                              | 5                             | 8.5                                 |
| Measuring range (µM) | 27-1056                      | 18-1768                      | 10-2000                      | 45-884                         | 18-2122                       | 18-1768                             |
| eGFR Calculation     | Yes                          | No                           | Yes                          | Yes                            | No                            | No                                  |

A= arterial C= capillary V= venous Syr= syringe

The ideal POCT creatinine meter will require only capillary sampling, fast turn-around-time of results and automatic eGFR calculation. In addition it should also have good analytical performance specifications in order to correctly categorise CKD risk and/or stages. (Adapted from Shephard, 2011)

## **1.4 PROJECT OVERVIEW**

### **1.4.1 Rationale for the study**

This study aimed to bridge the gap in knowledge with regard to the cut-off values for creatinine level measurements, and eGFR values, when screening a mixed ancestry populations. Currently, there is only documented and standardized cut-offs available for Caucasian and African American populations.

This study focused on the African mixed ancestry population and acted as a starting point for standardizing POCT creatinine cut-offs for other international mixed ancestry populations. Through the standardization of cut-offs for mixed ancestry populations, more accurate screening results can be obtained for patients suffering from diseases, which can lead to them receiving early treatment due to their early diagnosis. This in turn will help alleviate some of the disease and financial burden on developing countries' health systems because disease progression will be slowed and expensive aggressive treatments avoided.

### **1.4.2 Objectives of the study**

This study was a comparative study that was designed to evaluate the Nova Statsensor® point of care instrument for the measurement of creatinine for the detection of CKD in adult mixed ancestry subjects from the Bellville South Community in South Africa. Secondary objectives included (1) the prevalence of CKD based on the results of the instrument, and (2) the correlation between the Nova Statsensor®, and the central laboratory creatinine values (IDMS traceable). Ancillary objectives of the study were to evaluate the technical quality of POCT for creatinine in a community setting, as well as the evaluation of the cost implications when introducing this form of POCT into a primary care setting.

### **1.4.3 Research Question**

Can a POCT device such as the Nova Statsensor® correctly identify subjects with CKD when compared with a laboratory method that is IDMS traceable? What is the clinical and diagnostic accuracy of the Nova Statsensor® when screening the Bellville South mixed ancestry population. What are the possible cost implications of implementing such a device in local clinics?

### **1.4.4 Hypothesis**

The South African mixed ancestry population has a high prevalence of CKD due to studies showing a high prevalence of CKD risk factors such as diabetes mellitus, obesity, and hypertension. Implementation of a POCT device will allow for increased number of patients screened for CKD thus allowing for early detection and intervention in CKD treatment, and can also be used to monitor the disease progression.

The Nova Statsensor® displays satisfactory accuracy when compared to the IDMS traceable reference method. It shows good specificity for eliminating patients with eGFR values above 60 mL/min/1.73m<sup>2</sup>, thus making them a non-risk patient for CKD. This means that the POCT will perform adequately in detecting true negative results thus eliminating patients without CKD and prevent further expensive laboratory testing.

Implementation of a POCT device will allow for increased number of patients screened for CKD thus leading early detection and intervention in CKD treatment, end-stage renal failure, and renal replacement therapy. There will be high costs involved in implementation of such a device.

# **CHAPTER 2**

## **Research Methodology**



## 2.1 INTRODUCTION

The study was a comparative study that was designed and aimed to evaluate the performance of the Nova Statsensor® point of care instrument, through the measurement of creatinine, for the detection of CKD in adult mixed ancestry subjects from the Bellville South Community in South Africa. Secondary objectives included (1) the prevalence of CKD based on the results of the POCT instrument, and (2) the correlation between the Nova Statsensor®, and the central laboratory creatinine values (IDMS traceable). Ancillary objectives to the study evaluating the technical quality of POCT for creatinine in a community setting, as well as evaluating the cost implications involved when introducing POCT, for creatinine, into a primary care setting.

### 2.1.1 Selection of Nova Statsensor®

The Nova Statsensor® was previously evaluated in the local laboratory in the division of Chemical Pathology, Tygerberg hospital.

The primary aim was to compare POCT creatinine measurements produced by the POCT Nova Statsensor® Creatinine Meter with a laboratory based creatinine measurement performed with the Siemens ADVIA® 1800, which used an IDMS standardised kinetic Jaffe assay method.

The Nova Statsensor® Creatinine Meter was manufactured by Nova Biomedical and makes use of amperometric enzymatic biosensor technology.

This study made use of the MDRD equation as it measures eGFR directly as opposed to the CG-equation which first calculates CrCl. In addition to this, the MDRD equation is able to be adjusted for race therefore making it a better candidate as a starting point for developing standardized cut offs for ethnic groups other than Caucasians and African Americans.

A secondary aim was to assess the performance of the creatinine meter in a clinical setting and to assess its diagnostic utility when comparing the eGFR (4-variable MDRD) rate and renal disease staging.

This study involved a group of 45 healthy volunteer patients, attending the Nephrology outpatient clinic (Tygerberg Hospital). In this case, “healthy volunteer” refers to their overall health status excluding their kidney function. The finger prick method was used to obtain capillary blood sample for creatinine measurement on the Nova Statsensor® Creatinine POCT Meter. These results were compared to laboratory based serum creatinine method, performed on the Siemens ADVIA® 1800, which measured creatinine in venous blood samples. Both capillary and venous blood samples were collected at the same time.

The diagnostic accuracy was determined by comparing the calculated eGFR and different stages of renal disease, based on the KDIGO guidelines.

## 2.2 METHODS AND MATERIALS

### 2.2.1 Setting

The study formed part of the Bellville South, South Africa, Study (Matsha *et al*, 2013), which was investigating the prevalence of the independent risk factors for cardiovascular disease in the mixed ancestry population of Bellville South in the Western Cape.

According to 2011 census data (City of Cape Town Metropolitan Municipality 199 from Census 2011: <https://census2011.adrianfrith.com/place/199>), the Bellville South Community is a stable lower to middle income group of approximately 22000 people, which makes up 76 % of the total

population. Approximately 33 % (7200) of the mixed ancestry population is between the age of 35 and 65.

### 2.2.2 Sampling and sample size

Recruitment of participants from the mixed ancestry population in Bellville South, Ward 9, resulted in a stratified random sampling which included participants between the ages of 35 and 65.

Sample size determination for this study was done using the Australian National Statistical Service sample size calculator. A sample size of 845 participants, out of a total at-risk population of approximately 7200, was calculated. This conforms to a confidence level of 95% with a 3% confidence interval, and an estimated prevalence of the disease of approximately 26% in the population with a 90% response rate from the anticipated participants. These rates have been obtained from the USA KEEP surveys, in which CKD prevalence rates were estimated using a POCT instrument for creatinine measurement. Due to several logistical interferences, only 103 viable samples were obtained.

### 2.2.3 Sample Transport and Separation

Samples were collected and stored on ice while being transported by motor vehicle to the satellite Pathcare™ laboratory at Louis Leipoldt Hospital in Bellville, Cape Town. Once at the Pathcare™ laboratory, the blood samples were spun down through centrifugation in order to separate the blood serum from the cells. Once separated, the serum samples were then transported, by motor vehicle, at room temperature, to N1 City Pathcare™ research laboratory. The urine samples were transported in the same manner and to the same location, however these samples were stored on ice and not analysed until reaching the N1 City Pathcare™ research laboratory. Once samples had reached the Pathcare™ research laboratory creatinine analysis and urine albumin analysis were conducted on the relevant instruments.

### 2.2.4 Sample Analysis

The samples (venous) collected for this study were analysed in the Pathcare™ research laboratory, which is an accredited laboratory.

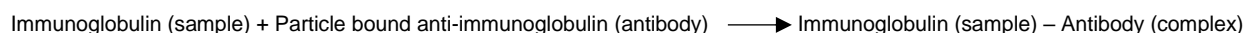
Venous blood samples' serums were analysed for creatinine measurement using a standardised creatinine assay traceable to IDMS performed on the Beckman Coulter AU5800 analyzer, at the N1 City Pathcare™ research laboratory.

The Beckman Coulter AU5800 measures creatinine using a kinetic modification of the Jaffe procedure. This involves the creatinine reacting with picric acid, at an alkaline pH, to form a yellow-orange complex. (Beckman Coulter AU5800 *creatinine product insert*)

Urine spot samples were analysed for albumin measurement using the Beckman Coulter, Inc, Image 800 analyzer. Analysis was carried out at the N1 City Pathcare™ research laboratory.

The Beckman Coulter Image 800 analyzer involves an antigen-antibody reaction with two forms of measurements: the Rate Nephelometry and Rate Turbidimetry systems.

The chemical reaction is as follows:





The Rate Nephelometry system measures the increase in the intensity of light that is scattered by particles present in the sample that is in a cuvette. The light source is a 670 nm laser. There is a detector that is placed at a 90° angle from the laser beam, in order to measure the light scatter. .

The Rate Turbidimetry system measures the decrease in the intensity of light that is scattered by the particles within the sample that is in the cuvette. The light source for this method is a light emitting diode with a wavelength of 940 nm. Detection occurs at 0° from the incident beam. (Beckman Coulter Immage *microalbumin product insert*)

### **2.2.5 Measurements**

Capillary blood finger prick samples, from fasting participants, were obtained by the trained personnel (Ms Tammy Krige), and measured on the Nova Statsensor® according to the manufacturer's guidelines. The results were documented on the "Patient Result Log" sheets.

Venous blood specimens were taken by the clinical staff for creatinine measurement by the central laboratory. The central laboratory used a method that is traceable to the IDMS gold standard reference method.

In conjunction to the capillary and venous blood samples, a urine sample was obtained from each participant in order to measure albumin levels by the conventional laboratory, which made use of the Beckman Coulter AU5800. The Beckman Coulter AU5800 used a kinetic modified Jaffe method.

### **2.2.6 Training**

All personnel carrying out any sample collection were trained in the specific field of sampling method, such as venous and capillary blood sampling. All personnel handling the POCT device were also trained for the regular maintenance and quality control procedures of the device.

Protocols for standard point of care operations at the study site were established prior to project commencement.

### **2.2.7 Quality Control**

Quality control (QC) was conducted according to the manufacturer's guidelines. For the purposes of the study, controls were run on each day that the instrument was used.

### **2.2.8 Clinical and Laboratory Measurements**

All consenting participants received a standardised interview and physical examination during which their blood pressure were measured according to the World Health Organisation (WHO) guidelines (Chalmers *et al.* 1999). This was done using a semi-automatic digital blood pressure monitor (Rossmax PA, USA) on the right arm, while the participant remained in a sitting position.

Other clinical measurements included the participant's body weight, height, waist and hip circumferences. Weight (to the nearest 0.1 kg) was determined while the subject was wearing light clothing and without shoes and socks.

An Omron Karada Scan Body Monitor scale was used to measure weight and body mass index. The scale was calibrated and standardized using a weight of known mass.

Height was measured using a Seca stadiometer. Waist circumference was measured using a non-elastic tape at the level of the narrowest part of the torso, as seen from the anterior view.

All anthropometric measurements were performed three times and the averaged value was used for analysis.

Fasting venous samples for various biochemical parameters (as part of the Bellville South, South Africa, Study) were obtained through the use of the OGTT. Morning spot urine samples were obtained for microalbumin measurements.

### **2.2.9 Laboratory Measurements**

Fasting blood samples were analysed for creatinine. These samples were measured using the standardised creatinine enzymatic assay traceable to IDMS performed on the Cobas 6000, manufactured by Roche.

Urine samples were analysed for albumin:creatinine ratio using the immunoturbidimetric assay performed on the Cobas 6000.

### **2.2.10 Statistical analysis**

Data was captured on the Microsoft Excel 2007 programme where further statistical analysis for method comparison, involving linear regression and Bland-Altman plots, was performed using the Analyse-It® statistical software version 2.3.

Diagnostic accuracy, which involved sensitivity, specificity positive predictive value (PPV) and negative predictive values (NPV), were calculated to assess the clinical utility of the Nova Statsensor® Creatinine POCT Meter. KDIGO Kidney disease stages  $\geq 3$  was used as the cut-off for CKD. This equates to an eGFR of  $<60 \text{ mL/min/1.73m}^2$  for an individual to be classified as having CKD.

### **2.2.11 Ethics**

The study was conducted according to the Declaration of Helsinki, and the South African Department of Health's 2004 Guidelines: *Ethics in Health Research: Principles, Structures, and Processes*. Specific oral and written consent by the participants was obtained. Participant information and informed consent forms were provided and completed by all participants. The forms included all relevant information regarding the current Bellville South, South Africa, Study research project. Ethics approval can be found in Appendix A.

## **2.3 SPECIFICATIONS OF THE NOVA STATSENSOR POINT OF CARE INSTRUMENT FOR CREATININE AND EGFR MEASUREMENTS**

### **2.3.1 Nova Statsensor® Creatinine Meter Specifications**

Acceptable Samples: Whole Blood: Capillary, Arterial, and Venous

Measurement Range: Creat: 0.30 – 12.0 mg/dL or 27 – 1056  $\mu\text{mol/L}$

Test Methodology: Electrochemistry

Measuring Technology: Enzyme, Amperometric

Sample Volume: 1.2  $\mu\text{L}$

Test Time: 30 seconds

Meter Memory: 1000 patient tests, 200 QC tests, 4000 Operator profiles

Battery: Rechargeable Li-polymer 3.7V 1800 mAh

Power: 3.7 V Li-polymer battery (Rechargeable/Replaceable)

Docking/Charging Station: Desk mount, Input: 100-240 V~, 50-60 Hz, 0.6A, Output: +12 V, 0.8 A

Data Output Port: RJ-45 Ethernet (10 Mbit)

Connectivity: Protocol TCP/IP Ethernet, Standard POCT1-A Compliant

Electrical Compliance: Meets IEC 61010, UL, and CSA standards

Dimensions: 153 mm x 82.5 mm x 46 mm

Weight: 360 g

### 2.3.2 Environmental Specifications

Temperature Range: 15 °C – 40 °C

Altitude: 4500 meters

Relative Humidity: Up to 90 % (noncondensing)

### 2.3.3 Chemistry Measurement Specifications

Sealed Test Strip and QC Stability: 12 months (4-8 °C)

Test Strips: 25 strips per vial

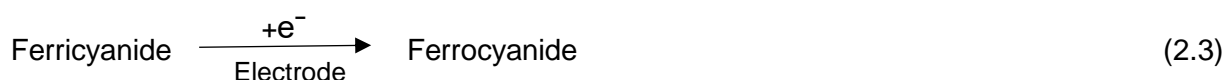
QC: three levels (low, normal, high)

**Table 2.1:** Quality control reagents' level and concentration.

| Control Level | Creatinine Levels (µmol/L) | CV % |
|---------------|----------------------------|------|
| Low           | 88                         | 8    |
| Medium        | 440                        | 6    |
| High          | 880                        | 4    |

### 2.3.4 Creatinine Measurement Methodology

The creatinine measurement is based on the following methodology:



The current generated at the electrode is proportional to the creatinine concentration of the sample. (Nova Biomedical user manual, 2012)

#### i) eGFR Determination

The below equation (2.4) will be used by the POCT device to calculate eGFR values of participants. This calculation involves the measurement of creatinine levels in participants. This will be done using the 4-Variable Modification of Diet in Renal Disease (MDRD-4) equation [Levey et al., 1999; 2006] which is applicable to standardised serum creatinine values without ethnicity adjustment for all participants. Staging of kidney function will be based on the National Kidney Foundation's

Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) classification (Levey *et al.* 2003). An eGFR < 60 mL/min will be used to define chronic kidney disease (or CKD stage 3-5) as previously seen in table 1.1.

The Statsensor® Creatinine Hospital Meter is plasma calibrated and can use several creatinine estimation equations: MDRD, IDMS traceable MDRD, Schwartz, Counahan-Barratt and Cockcroft-Gault. (Nova Biomedical user manual, 2012)

For this study, the modified MDRD-SI equation, which uses the SI creatinine Units  $\mu\text{mol/L}$  will be used. The MDRD-SI equation is as follows:

$$\text{GFR MDRD (mL/min/1.73m}^2\text{)} = 186 \times [\text{SCr}/88.4]^{-1.154} \times [\text{age}]^{-0.203} \times [0.742 \text{ if female}] \times [1.210 \text{ if African American}] \quad (2.4)$$

The above equation requires 4 variables:

- i. Serum, or plasma, creatinine (SCr) in  $\mu\text{mol/L}$
- ii. Age in years (18 years to 130 years)
- iii. Sex (Male or Female)
- iv. Race (African American or “All other races”)

## 2.4 VALIDATION OF THE NOVA STATSENSOR CREATININE HOSPITAL METER

The manufacturers of the Nova Statsensor® Creatinine Hospital Meter have performed an in-house method validation, where the instrument displayed satisfactory performance. This information is available in Appendix B.

# **CHAPTER 3**

## **Results and Discussion**

### 3.1 RESULTS

#### 3.1.1 Prevalence of CKD and CKD status

**Table 3.1:** Summary of subjects and relevant information.

| Sample ID | Age (Years) | Gender | Diagnosis (SEMSDA) | POC Creatinine (umol/L) | Lab test: CREATININE-S* (umol/L) | POC eGFR (mL/min/1.73 m2) | Lab test: MDRD (GFR ESTIMATE) (ml/min) | Stage CKD Lab | Stage CKD StaSensor (KDIGO) |
|-----------|-------------|--------|--------------------|-------------------------|----------------------------------|---------------------------|--|---------------|-----------------------------|
| WC9-A1860 | 52          | F      | Known DM           | 42                      | 52                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1861 | 38          | M      | Normal             | 106                     | 71                               | 72                        | > 90                                   | 1             | 2                           |
| WC9-A1862 | 59          | M      | Normal             | 71                      | 93                               | >90                       | 72                                     | 2             | 1                           |
| WC9-A1863 | 48          | M      | Normal             | 42                      | 58                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1864 | 73          | F      | IGT                | 64                      | 59                               | 84                        | 86                                     | 2             | 2                           |
| WC9-A1865 | 42          | F      | Normal             | 43                      | 53                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1866 | 46          | F      | Normal             | 42                      | 52                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1867 | 74          | F      | IGT                | 55                      | 64                               | >90                       | 79                                     | 2             | 1                           |
| WC9-A1868 | 67          | F      | Normal             | 65                      | 50                               | 84                        | > 90                                   | 1             | 2                           |
| WC9-A1869 | 60          | F      | IGT                | 53                      | 56                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1870 | 68          | F      | Normal             | 61                      | 69                               | 90                        | 83                                     | 2             | 1                           |
| WC9-A1871 | 73          | M      | Normal             | 72                      | 90                               | >90                       | 71                                     | 2             | 1                           |
| WC9-A1872 | 54          | M      | Normal             | 58                      | 57                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1873 | 39          | M      | Normal             | 80                      | 77                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1874 | 22          | M      | Normal             | 48                      | 61                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1875 | 52          | F      | Normal             | 57                      | 62                               | >90                       | 88                                     | 2             | 1                           |
| WC9-A1876 | 58          | F      | Normal             | 35                      | 41                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1877 | 29          | F      | Normal             | 58                      | 43                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1878 | 45          | F      | Normal             | 43                      | 46                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1879 | 67          | F      | IGT                | 45                      | 58                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1880 | 65          | F      | Normal             | 51                      | 63                               | >90                       | 82                                     | 2             | 1                           |
| WC9-A1881 | 38          | F      | Normal             | 63                      | 51                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1882 | 42          | F      | Normal             | 54                      | 56                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1883 | 46          | F      | IGT                | 70                      | 69                               | 83                        | 80                                     | 2             | 2                           |
| WC9-A1884 | 58          | F      | Normal             | 59                      | 65                               | >90                       | 81                                     | 2             | 1                           |
| WC9-A1885 | 30          | F      | Normal             | 50                      | 57                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1886 | 80          | F      | Normal             | 57                      | 66                               | >90                       | 75                                     | 2             | 1                           |
| WC9-A1887 | 43          | F      | Normal             | 61                      | 60                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1888 | 60          | F      | Normal             | 46                      | 54                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1891 | 71          | F      | IGT                | 72                      | 55                               | 74                        | > 90                                   | 2             | 2                           |
| WC9-A1892 | 61          | F      | IGT/IFG            | 58                      | 60                               | >90                       | 88                                     | 1             | 2                           |
| WC9-A1893 | 63          | F      | Normal             | 70                      | 58                               | 78                        | > 90                                   | 2             | 1                           |
| WC9-A1894 | 54          | M      | Normal             | 59                      | 70                               | >90                       | > 90                                   | 1             | 2                           |
| WC9-A1895 | 27          | M      | Normal             | 50                      | 61                               | >90                       | > 90                                   | 1             | 1                           |
| WC9-A1896 | 74          | M      | IGT                | 263                     | 275                              | 22                        | 19                                     | 1             | 1                           |

|           |    |   |          |     |     |     |      |    |    |
|-----------|----|---|----------|-----|-----|-----|------|----|----|
| WC9-A1898 | 60 | F | Known DM | 60  | 57  | >90 | > 90 | 4  | 4  |
| WC9-A1900 | 56 | M | IGT      | 94  | 90  | 77  | 69   | 1  | 1  |
| WC9-A1901 | 68 | F | IGT      | 57  | 65  | >90 | 79   | 2  | 2  |
| WC9-A1902 | 58 | M | IGT      | 49  | 47  | >90 | > 90 | 2  | 2  |
| WC9-A1903 | 49 | M | Normal   | 67  | 69  | >90 | > 90 | 2  | 1  |
| WC9-A1904 | 86 | M | IGT      | 79  | 91  | 86  | 68   | 1  | 1  |
| WC9-A1905 | 44 | F | DM       | 65  | 68  | >90 | 81   | 1  | 1  |
| WC9-A1906 | 59 | M | Normal   | 57  | 65  | >90 | > 90 | 2  | 2  |
| WC9-A1907 | 57 | M | Normal   | 64  | 68  | >90 | > 90 | 2  | 1  |
| WC9-A1908 | 57 | M | Normal   | 140 | 129 | 48  | 49   | 1  | 1  |
| WC9-A1909 | 55 | F | Normal   | 49  | 64  | >90 | 83   | 1  | 1  |
| WC9-A1910 | 46 | F | IGT      | 39  | 60  | >90 | > 90 | 3a | 3a |
| WC9-A1911 | 51 | F | Known DM | 59  | 69  | >90 | 78   | 2  | 1  |
| WC9-A1912 | 54 | F | Normal   | 42  | 50  | >90 | > 90 | 1  | 1  |
| WC9-A1913 | 36 | F | Normal   | 44  | 57  | >90 | > 90 | 2  | 1  |
| WC9-A1914 | 39 | F | Normal   | 55  | 63  | >90 | > 90 | 1  | 1  |
| WC9-A1915 | 47 | F | Known DM | 49  | 55  | >90 | > 90 | 1  | 1  |
| WC9-A1916 | 59 | F | Known DM | 76  | 100 | >90 | 66   | 1  | 1  |
| WC9-A1917 | 59 | F | Normal   | 44  | 58  | >90 | > 90 | 1  | 1  |
| WC9-A1918 | 59 | F | Normal   | 62  | 56  | >90 | > 90 | 2  | 1  |
| WC9-A1919 | 55 | F | Normal   | 34  | 46  | >90 | > 90 | 1  | 1  |
| WC9-A1920 | 58 | F | Normal   | 56  | 61  | >90 | 88   | 1  | 1  |
| WC9-A1921 | 54 | F | Normal   | 50  | 54  | >90 | > 90 | 1  | 1  |
| WC9-A1922 | 58 | F | Normal   | 47  | 47  | >90 | > 90 | 2  | 1  |
| WC9-A1923 | 27 | F | Normal   | 50  | 46  | >90 | > 90 | 1  | 1  |
| WC9-A1925 | 57 | F | Normal   | 58  | 62  | >90 | 87   | 1  | 1  |
| WC9-A1926 | 52 | M | IGT      | 79  | 82  | >90 | 84   | 1  | 1  |
| WC9-A1928 | 58 | M | Normal   | 65  | 68  | >90 | > 90 | 2  | 1  |
| WC9-A1929 | 56 | M | Normal   | 72  | 58  | >90 | > 90 | 2  | 1  |
| WC9-A1935 | 55 | F | Known DM | 59  | 44  | >90 | > 90 | 1  | 1  |
| WC9-A1936 | 64 | F | Known DM | 47  | 48  | >90 | > 90 | 1  | 1  |
| WC9-A1937 | 20 | F | Normal   | 54  | 43  | >90 | > 90 | 1  | 1  |
| WC9-A1938 | 30 | M | Normal   | 72  | 67  | >90 | > 90 | 1  | 1  |
| WC9-A1939 | 49 | F | IGT      | 75  | 53  | 76  | > 90 | 1  | 1  |
| WC9-A1940 | 23 | M | Normal   | 60  | 47  | >90 | > 90 | 1  | 1  |
| WC9-A1941 | 42 | M | Normal   | 61  | 65  | >90 | > 90 | 1  | 1  |
| WC9-A1942 | 39 | F | Normal   | 50  | 50  | >90 | > 90 | 1  | 2  |
| WC9-A1943 | 45 | M | Normal   | 48  | 50  | >90 | > 90 | 1  | 1  |
| WC9-A1944 | 53 | F | Normal   | 60  | 59  | >90 | > 90 | 1  | 1  |
| WC9-A1945 | 55 | M | Known DM | 72  | 104 | >90 |      | 1  | 1  |
| WC9-A1946 | 60 | F | Normal   | 64  | 65  | 87  | 81   | 1  | 1  |

|           |    |   |          |    |    |     |      |    |   |
|-----------|----|---|----------|----|----|-----|------|----|---|
| WC9-A1947 | 61 | M | IGT      | 79 | 98 | >90 | 67   | 1  | 1 |
| WC9-A1949 | 34 | F | Normal   | 66 | 69 | >90 | 85   | 2  | 2 |
| WC9-A1950 | 32 | F | Normal   | 50 | 50 | >90 | > 90 | 2  | 1 |
| WC9-A1951 | 45 | F | Normal   | 46 | 47 | >90 | > 90 | 2  | 2 |
| WC9-A1952 | 47 | M | Normal   | 48 | 72 | >90 | > 90 | 2  | 1 |
| WC9-A1953 | 57 | F | Normal   | 42 | 60 | >90 | 90   | 1  | 1 |
| WC9-A1954 | 61 | M | Normal   | 54 | 88 | >90 | 76   | 1  | 1 |
| WC9-A1955 | 23 | F | Normal   | 37 | 48 | >90 | > 90 | 1  | 1 |
| WC9-A1956 | 34 | F | Normal   | 43 | 53 | >90 | > 90 | 1  | 1 |
| WC9-A1957 | 24 | F | Normal   | 47 | 47 | >90 | > 90 | 2  | 1 |
| WC9-A1961 | 64 | F | Known DM | 42 | 61 | >90 | 86   | 1  | 1 |
| WC9-A1962 | 65 | M | Known DM | 40 | 81 | >90 | 83   | 1  | 1 |
| WC9-A1963 | 44 | F | Known DM | 45 | 63 | >90 | 88   | 1  | 1 |
| WC9-A1964 | 57 | F | Normal   | 40 | 60 | >90 | 90   | 2  | 1 |
| WC9-A1965 | 38 | F | Normal   | 46 | 60 | >90 | > 90 | 2  | 1 |
| WC9-A1966 | 66 | F | DM       | 49 | 72 | >90 | 70   | 2  | 1 |
| WC9-A1967 | 52 | F | Normal   | 49 | 68 | >90 | 79   | 1  | 1 |
| WC9-A1968 | 65 | M | DM       | 46 | 78 | >90 | 86   | 1  | 1 |
| WC9-A1969 | 60 | F | Normal   | 54 | 70 | >90 | 75   | 2  | 1 |
| WC9-A1970 | 61 | F | Normal   | 58 | 70 | >90 | 74   | 2  | 1 |
| WC9-A1971 | 64 | F | DM       | 31 | 57 | >90 | > 90 | 2  | 1 |
| WC9-A1972 | 68 | F | Normal   | 41 | 88 | >90 | 56   | 2  | 1 |
| WC9-A1973 | 53 | F | Normal   | 50 | 53 | >90 | > 90 | 2  | 1 |
| WC9-A1974 | 54 | M | Normal   | 53 | 84 | >90 | 82   | 1  | 1 |
| WC9-A1975 | 42 | M | Normal   | 54 | 68 | >90 | > 90 | 3a | 1 |
| WC9-A1980 | 55 | M | Normal   | 50 | 66 | >90 | > 90 | 1  | 1 |

DM = Diabetes mellitus IGT= Impaired glucose tolerance IFG= Impaired fasting glucose

**Table 3.2:** Percentage male and female participants and prevalence of diabetes mellitus, impaired glucose tolerance, and impaired fasting glucose within the study group (n=103).

| Gender                      | Number of participants | Prevalence (%) |
|-----------------------------|------------------------|----------------|
| Female                      | 71                     | 68.93          |
| Male                        | 32                     | 31.07          |
| Total                       | 103                    | 100            |
| Health status               |                        |                |
| Normal health               | 73                     | 70.87          |
| Known Diabetes mellitus     | 10                     | 9.71           |
| Diagnosed Diabetes mellitus | 4                      | 3.88           |
| IGT                         | 15                     | 14.56          |
| IGT and IFG                 | 1                      | 0.97           |
| Total                       | 103                    | 100            |

IGT = impaired glucose tolerance IFG= impaired fasting glucose



**Table 3.3:** Agreement of CKD status between the POCT device and laboratory reference method for the three participants, from the study group n=103, who tested positive for stage 3a to stage 5 CKD.

| Participant (n=3)                        | Age | Gender | Health | POCT Nova Statsensor® |                                    |           | Laboratory reference method |                                    |           |
|--|-----|--------|--------|-----------------------|------------------------------------|-----------|-----------------------------|------------------------------------|-----------|
|  |     |        |        | Creatinine (µmol/L)   | eGFR (mL/min /1.73m <sup>2</sup> ) | CKD stage | Creatinine (µmol/L)         | eGFR (mL/min /1.73m <sup>2</sup> ) | CKD stage |
| WC9-A1898                                | 60  | female | DM     | 54                    | >90                                | 3a        | 68                          | >90                                | 1         |
| WC9-A1910                                | 46  | female | IGT    | 39                    | >90                                | 3a        | 60                          | >90                                | 3a        |
| WC9-A1975                                | 42  | male   | Normal | 60                    | >90                                | 4         | 57                          | >90                                | 4         |
| <b>Overall prevalence of CKD = 2.91%</b> |     |        |        |                       |                                    |           |                             |                                    |           |

**Table 3.4:** Overall agreement of results between the POCT device and the laboratory reference method, displayed as a percentage of the number of aligned results out of the total of 103 participants' results.

|                   | POCT aligned with laboratory reference method (% that aligned) | POCT non-alignment with laboratory reference method (% that did not aligned) | Over-estimation of value (%) | Under-estimation of value (%) |
|-------------------|--|--|------------------------------|-------------------------------|
| Creatinine values | 2.88   | 96.12  | 34.95                        | 61.17                         |
| eGFR values       | 55.34  | 44.66  | 37 of 103                    | 8.74                          |
| CKD staging       | 66.99  | 33.01  | 29 of 103                    | 4.85                          |

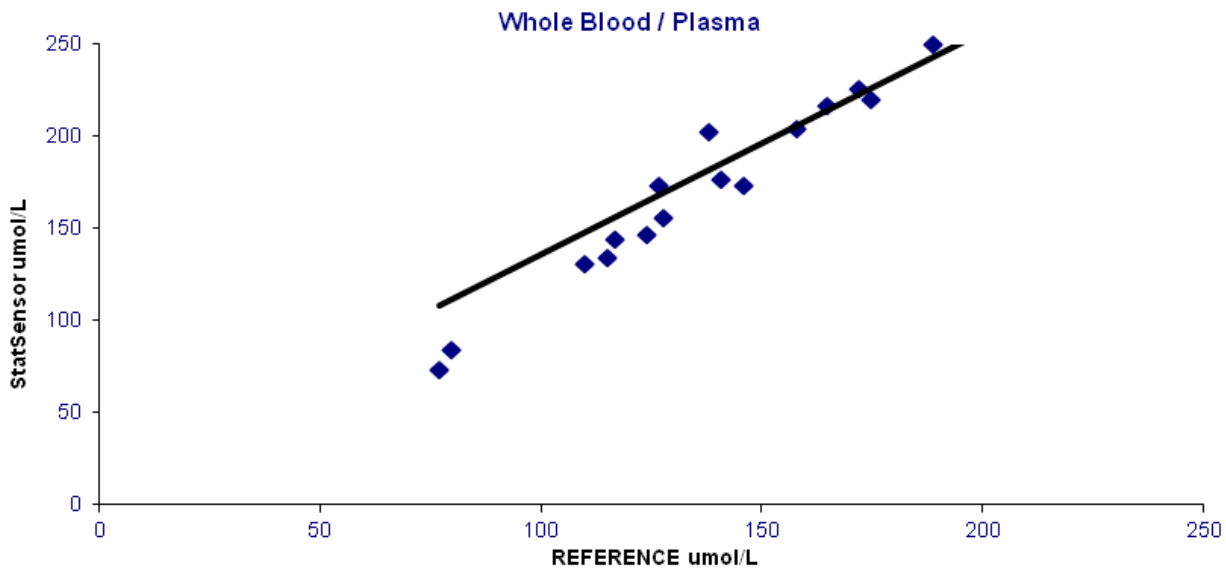
Table 3.4 deals with the overall agreement of results between the POCT Statsensor® results and the laboratory method.

### 3.1.2 Laboratory evaluation

Imprecision (coefficient of variance, CV %) on the POCT device was evaluated using repeated analysis of two levels of Nova Statsensor® quality control (QC) material. The accuracy of creatinine results obtained with Nova Statsensor® device was compared to the results obtained with the laboratory reference analyzer (Siemens ADVIA® 1800) using spiked (different creatinine concentrations) donor heparinised venous blood samples. This analysis was carried out by Dr Megan Rensburg.

**Table 3.5:** Imprecision evaluation of the POCT Nova Statsensor® compared to the laboratory reference analyser (Siemens ADVIA® 1800) .

|                 | Control level 1 (n=20)<br>44-124 µmol/l | Control level 2 (n=20)<br>398-663 µmol/l |
|-----------------|---|--|
| Within-day CV%  | 5.3                                     | 0.8                                      |
| Between-day CV% | 4.4                                     | 2.4                                      |

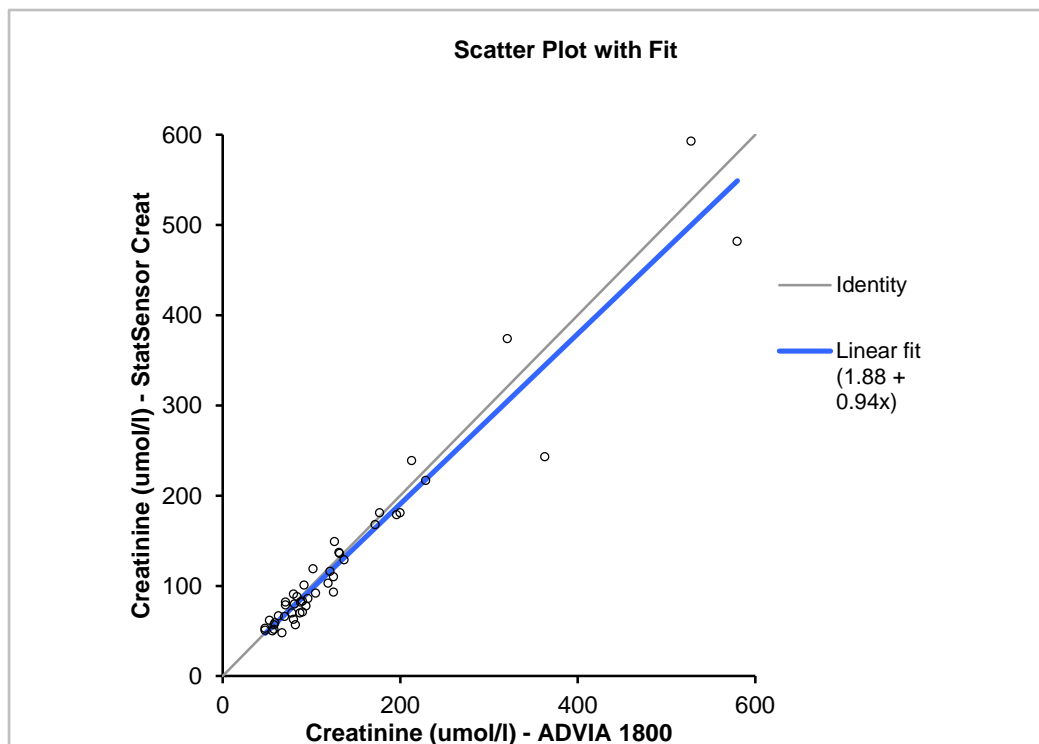


$$y = 1.2x + 14.8 \quad R^2 = 0.98$$

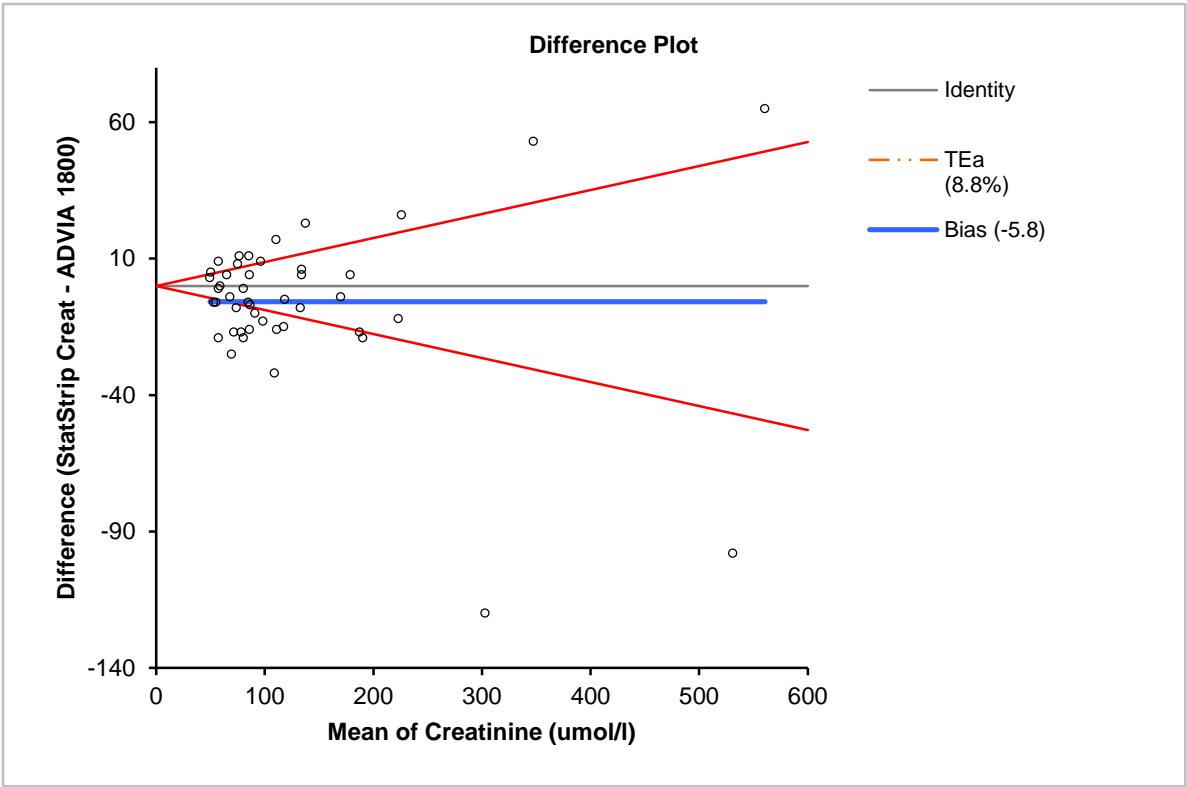
**Figure 3.1:** POCT device accuracy versus the laboratory reference method.

### 3.1.3 Clinical setting

#### 3.1.3.1 Renal Clinic Patients



**Figure 3.2:** Linear regression curve between the Nova Statsensor® and reference method, ADVIA® 1800.

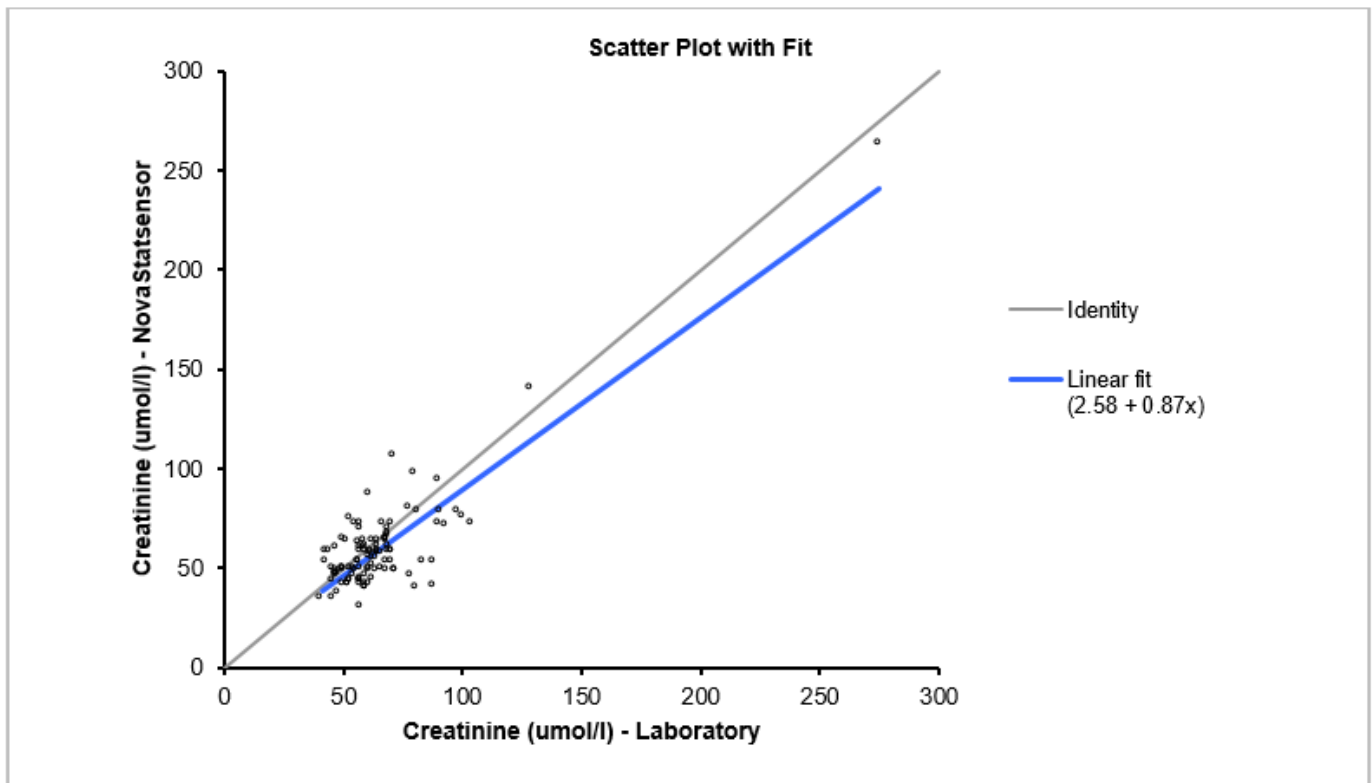


**Figure 3.3:** Bland-Altman plot depicting total allowable error (Westgard guidelines, <https://www.westgard.com/biodatabase1.htm>)

**Table 3.6** Diagnostic accuracy results of the clinical setting evaluation.

| ≥ Stage 3 CKD | %     |
|---------------|-------|
| Sensitivity   | 66.67 |
| Specificity   | 100   |
| PPV           | 100   |
| NPV           | 99    |

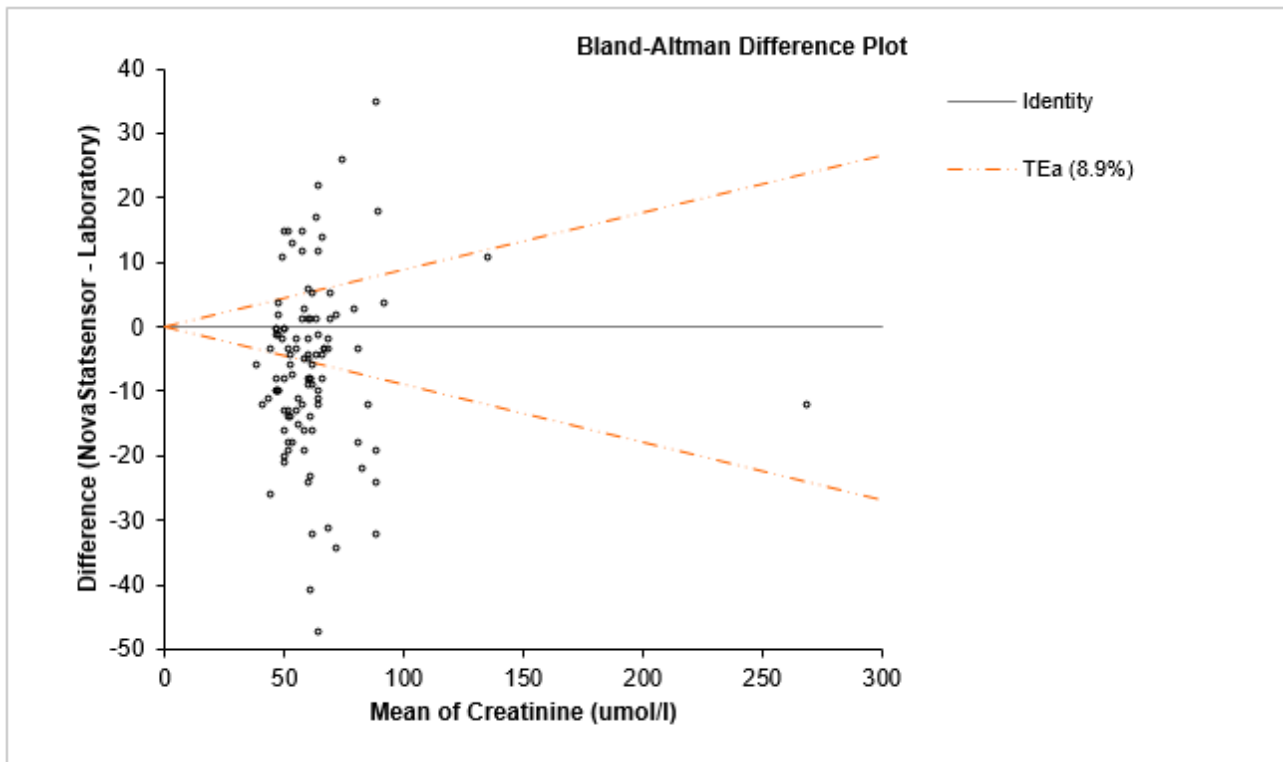
### 3.1.4 Bellville South community participants results



**Figure 3.4:** Scatter plot depicting the correlation between the Nova Statsensor® POCT device and the laboratory reference method (Bellville-South Project).

According to figure 3.4, there is negative proportional bias for the Nova Statsensor®.

### 3.1.4.1 Method comparison



**Figure 3.5:** Bland-Altman difference plot depicting total allowable error.

Figure 3.5 demonstrates that there is a total allowable error (TEa) of 8.9%, based on the Westgard Guidelines, with creatinine biological variation within-subjects being 14.7 and between-subjects 2.98. In this Bland-Altman graph, which measures the agreement between the two methods, one can see that the larger the mean creatinine value, the larger the range of total allowable error. In this graph it appears that more than half the values fall outside of the TEa range.

### 3.1.5 Diagnostic accuracy

**Table 3.7:** Diagnostic accuracy calculated using eGFR values and subsequent staging of CKD, with the laboratory method as the reference method.

| ≥ Stage 3 CKD      | %     |
|--------------------|-------|
| <b>Sensitivity</b> | 66.67 |
| <b>Specificity</b> | 100   |
| <b>PPV</b>         | 100   |
| <b>NPV</b>         | 99    |

Table 3.7 displays excellent diagnostic accuracy when looking at eGFR values and CKD staging.

### 3.1.6 Cost implications of introducing the Nova Statsensor® into primary care

Introducing such a device into a primary care setting within a community will initially be extremely expensive. It will involve buying stock consisting of test strips, control solutions, and also involve buying the device itself. Based on this study, the expenses involved in introducing this method of testing are presented in the below table 3.8. This data was generated by Ms Tammy Krige.

On average the NHLS laboratory at Tygerberg Hospital receives 22 444 number of requests for patient Creatinine testing monthly. One must consider adding additional tests in order to cover QC testing and patient re-testing. Based on the information provided by the NHLS laboratory at Tygerberg Hospital, this brings the total approximate number of monthly creatinine tests conducted to 22 970.

**Table 3.8:** Monthly expenses for Statsensor® Creatinine Meter consumables based on running 22 970 tests per month.

| Product   | Cost per unit (R) | Number of units required to cover 22970 creatinine tests per month | Cost for 1 month supply based on 22970 tests run per month (R) |
|---|-------------------|--|--|
| Nova Biomedical Statsensor® Creatinine Test Strips (50's) | 5 796.90          | 460  | 2 666 574  |
| Control Solution Level 1 (4mL bottle)                     | 191.84            | 1  | 191.84   |
| Control Solution Level 3 (4mL bottle)                     | 191.84            | 1  | 191.84   |
| Total   | 960.58            | -  | 2 666 957.68   |

Purchasing the Statsensor® Creatinine Meter KIT will be an initial once off expense, the total amount is included in table 3.9 as the first month's total expenses for implementation.

**Table 3.9:** Total costs involved in the first month of POCT implementation when the Statsensor® Creatinine Meter KIT is paid as a lump sum.

| Product  | Total cost (R) |
|--|----------------|
| Statsensor® Creatinine Connectivity Data Management Meter KIT (including Docking Station, power adapter, and rechargeable battery) | 104 344.20     |
| Month supply of consumables (as seen in table 3.8)   | 2 666 957.68   |
| Total  | 2 771 301.88   |

If, for example, a 24 month payment plan is in place, it will cost the State a relatively constant amount for each month. This amount will include the Statsensor® Creatinine Meter KIT fee and a month's supply of consumable stock, as seen in the below table 3.10, which may vary slightly for the odd month based on increased or decreased test demands.

**Table 3.10:** Monthly expenses involved in purchasing the Statsensor® Creatinine Connectivity Data Management Meter KIT with a 24 month payment plan.

| Product  | Cost per unit | Cost for 1 month supply based on 22970 tests run per month with payment plan of 24 months(R) |
|--|---------------|--|
| Nova Biomedical Statsensor® Creatinine Test Strips (50's)  | 5 796.90      | 2 666 574  |
| Control Solution Level 1 (4mL bottle)  | 191.84        | 191.84   |
| Control Solution Level 3 (4mL bottle)  | 191.84        | 191.84   |
| Statsensor® Creatinine Connectivity Data Management Meter KIT (including Docking Station, power adapter, and rechargeable battery) | 104 344.20    | 4347.68  |
| Total  | 2 771 301.88  | 2 666 957.68   |

Making use of a payment plan is especially feasible for developing countries when implementing POCT because it lessens the initial financial load on the health care system as a monthly budget can be set up in order to ensure even distribution of medical funds for throughout the year.

### 3.2 Discussion

The study investigated the prevalence of CKD based on the results of the Nova Statsensor® POCT instrument. The study also evaluated the correlation between the Nova Statsensor®, and the central laboratory creatinine values (IDMS traceable). Additionally, the technical quality of Nova Statsensor® POCT device, testing for creatinine, was assessed within a community setting. The study also evaluated the cost implications involved in implementing the POCT into a community care setting. The study results provide a CKD prevalence of 2.9% with the mixed ancestry study population. The study found that the creatinine value correlation between the POCT device method and the laboratory reference method, was very poor with only 3.89% of the POCT values correlating to the reference method results. The eGFR values had better agreement with 55.34% correlation between the POCT device measurement and the central laboratory measurement. The two methods correlated well in terms of CKD staging with 67% agreement. The study found a low sensitivity of only 66.7% and a high specificity of 100% for the Nova Statsensor® when compared against the laboratory reference method. The technical quality of the Statsensor® was satisfactory as it was user friendly. The cost of implementation of the Nova Statsensor® POCT device would cost the State R 2 771 301.88 for device implementation and 1 month supply of relevant consumables. This price would mostly be unaffordable for governments, especially those of developing countries.

The study group of 103 participants consisted mainly of females (68.9%) and the majority (70.9%) of participants had a health status of normal health, with no additional health issues. However 9.7% of the study group suffered from known, pre-existing DM. Through the study 3.9% were diagnosed with DM and it was also found that 14.6% of the participants suffered from IGT. A very small percentage, (0.97%) suffered from both IGT and IFG. These are all risk factors for developing CKD. Based on these figures, the prevalence of risk factors was 29.1% that is almost a third of the population at risk of developing CKD. This is considered a high prevalence within the mixed ancestry population, especially when considering

the Nigerian African population diabetes prevalence is 2.1% and 2.8% in the African Cameroonian populations. These results were found in studies conducted by Okoye *et al* (2011) and Kaze *et al* (2015) respectively.

The total prevalence of CKD within the study group tested by the POCT device was 2.9%, which can be statistically translated to: for every 100 mixed ancestry persons, three will suffer from CKD. The 2.9% prevalence finding of this study are considerably lower than that of the study conducted by Matsha *et al* (2013), where the prevalence was found to be 7.6% for MDRD equation adjusted for ethnicity and 23.9% when not adjusting for ethnicity. The difference in prevalence between the two studies could be due to the differences in sample size. Matsha *et al* (2013) had a final sample size of 1202 with 75.3% of participants being female and the average age being 52.9 years of age, compared to this study having only 103 participants and which had 68.9% females with an average age of 52.2 years of age. These dynamics within the sample groups may also partially account for the lower CKD prevalence in this study. Other possible influential dynamics may include the mean age of the sample population and difference in prevalence of CKD risk factors such as diabetes and hypertension. However in these two studies the mean ages were very similar therefore eliminating this dynamic as a possible influence of the difference in CKD prevalence. Similarly the prevalence of diabetes within both studies' populations were 26.4% for Matsha *et al* (2013) and 29.2% for this study. Again, these values are significantly similar therefore eliminating diabetes as an influential dynamic on the difference in studies' prevalence of CKD. The only major differing possible influential factors include sample size and gender composition of each study. Matsha *et al* (2013) had a much larger sample size comprising of mainly female participants (75.3%), whereas this study had a female population of only 68.93%. A study conducted by Eriksen and Ingebreetsen (2006) found that females had a higher prevalence of CKD than their male counterparts, this supports the finding by Matsha *et al* (2013) versus this study. In this study there was a population of 68.9% females whereas Matsha *et al* (2013) had a female population of 75.3%. This could have possibly led to the higher prevalence of CKD found in the study conducted by Matsha *et al* (2013).

The three participants, of the 103 subject population, that the POCT Nova Statsensor® determined to have CKD show good agreement between the POCT and laboratory testing methods when based on eGFR values. However when the creatinine values are assessed it is noted that Statsensor® underestimated the creatinine values in the female participants and overestimated the creatinine levels in the male participant, if only by 3  $\mu\text{mol/L}$ . The underestimation is a concern as it can lead to CKD positive patients obtaining a false negative result. Based on this table, the Statsensor® and laboratory reference method agree on two of the three CKD staging results of the listed participants. It was noted that in participant WC9-A1898 the gap between the stages produced by each method was notably large. The POCT device diagnosed stage 3a where the laboratory method diagnosed as stage 1. Again it is better to have an over estimation as this will lead to less patients receiving false negative results, and instead receive a false positive result which will lead to a referral for further testing thus confirming their health status. This is a good precautionary measure, unfortunately it may lead to increased unnecessary expenses for the government health sector. The total prevalence of CKD within the study group tested by the POCT device was 2.9%, which can be statistically translated to; for every 100 mixed ancestry persons, three will suffer from CKD. The laboratory method found a prevalence of only 1.94% due to participant WC9-A1898 receiving a false positive on the POCT device. This number can easily increase over time due to the high prevalence of CKD risk factors within the mixed ancestry population.

The overall agreement of results between the POCT Statsensor® results and the laboratory method show that the creatinine value agreement was very poor with only 3.89% of the POCT values correlating to the reference method results. The POCT Statsensor® also mainly under-estimated creatinine results, 61.17% underestimated, which is not desirable as this may possibly lead to false negatives. The eGFR values had better agreement, 55.34%, however these were mainly over estimated by the Statsensor®. This can again lead to false negatives. The two methods agreed well in terms of CKD staging with 67% agreement.



However it again over estimated some of the results therefore suggesting an over estimation of progression of CKD. This is not a massive concern as the result will most likely be false positives arising which will lead to further referrals thus preventing patients going untreated for CKD. Additional analysis using the Youden Index method and Receiving Operating Characteristics (ROC) curve can add additional insight into the agreement between the two methods, however this analysis was not performed during this study.

The comparability of the POCT device against the reference method could not be measured using creatinine values alone as the creatinine levels showed poor correlation between the two methods of measurement. Therefore one must rather make use of the MDRD equation for eGFR calculation which gives rise to improved correlation. This is because creatinine levels depend on several factors which include muscle mass, obesity, gender, and age. In addition, creatinine has a wide reference interval which also contributes to it being undesirable as a sole marker for measurement. The MDRD equation factors in these variables and thus adjusts the results to make them more relevant and accurate. There are several other eGFR equations that include IDMS traceable MDRD, Schwartz, and Cockcroft-Gault.

The Bland-Altman difference plot depicting total allowable error brings to question whether its results, with many values falling outside the TEa range, are due to analytical error or simply biological error, which may result in altering the final eGFR value from the POCT device. Possible biological interferences include several possibilities. The first being antibiotics such as aminoglycosides, particularly Streptomycin which has been found to almost double the apparent creatinine concentration, this can be exacerbated by the risk of possible nephrotoxic effects on the kidneys which will decrease kidney function and further increase the apparent creatinine concentration. Other antibiotics such as cephapirin, cefazolin, and cephalthrin have also been found to interfere with creatinine measurement at various concentrations (Syal *et al.* 2013). These compounds were not tested for in this study as Nova Biomedical states that there is negligible interference on the POCT device with regard to the former mentioned compounds. The second possible biological interferences include bilirubin, glucose, and acetone being present within the blood (Syal *et al.* 2013). These compounds have been found to affect the Jaffe method. This is important because in this study the reference analytical method was based on a Jaffe assay. This leads to the possibility that unexpected biological interferences could have occurred. The dispersion could also be due to the difference in analytical methods of creatinine measurement. The Nova Statsensor® makes use of electrochemistry to measure creatinine using an enzyme and amperometric measuring technology. The laboratory method is run on the Beckman Coulter AU5800 and makes use of a kinetic modified Jaffe method which is a colorimetric method. According to Nova Biomedical the Nova Statsensor® is IDMS traceable, therefore traceability of the POCT device is not an issue (Nova Biomedical Statsensor® pamphlet insert). Based on this graph it appears that there is sub-optimal agreement between the two methods.

According to the results there is excellent diagnostic accuracy when looking at eGFR values and CKD staging. A 100% specificity was recorded which is a desired result as this study aims to eliminate participants who test negative for CKD and have a eGFR value of above 60 ml/min/1.73m<sup>2</sup>, or are staged as stage 1 or 2. Screening programs work through elimination of healthy individuals. If an eGFR value is below 60 ml/min/1.73m<sup>2</sup> and staging is stage 3a or beyond, then the patient will be referred for further medical tests in order to make a definite diagnosis. Specificity and sensitivity and co-dependent values, therefore due to the high specificity, the POCT device has a lower sensitivity of 66.67%. These are acceptable results and are supported in a study conducted by Shephard *et al* (2010) where they found the specificity to also be 100% and the sensitivity to be 87%. However it must be taken into account that the low sensitivity of 66.67% indicates that the Statsensor® has a low ability to test for true positives, but when the high specificity is taken into account it can be concluded that the Statsensor® is more likely to produce false positives where patients without CKD are tested as positive. This is an acceptable characteristic as it will ensure that patients with existing CKD will not be missed. These results suggest that 33.33% of all

tests will test as false positive. In the case of this study, this translates to 34 participants receiving a false positive result. The problem with a third of all tests being false positive is that it will result in many referrals for further laboratory testing which may incur unnecessary expenses for the State.

The positive predictive value (PPV) and negative predictive value (NPV) are 100% and 99% respectively. Therefore one can 100% certain that a positive result is a true positive result, making it easy to identify patients who need further diagnostic testing for CKD status. The NPV value allows one to be 99% certain that a negative result on the POCT device is a true negative result, therefore the patient does not require further diagnostic testing. Statistically only 1 in 100 screened patients may receive a false negative result according to this NPV. These results seem to contradict the results of the 66.67% sensitivity resulting in roughly a third of all tests testing as false positives.

Overall, possible errors leading to the non-alignment of data could be due to pre-analytical errors where samples were not kept on ice during transit to laboratory, or possibly due to incorrect sampling procedure followed when using the POCT device (Plebani M. 2012). Non-alignment of results between the two methods can also be due to interferences in each one of the two methods. Interference of less than 20% can occur between 3 and 12g/dL of protein in the Beckman Coulter AU5800 reference method (Beckman Coulter Creatinine pamphlet insert), with no known significant interferences in the Statsensor® (Nova Biomedical Statsensor® creatinine meter pamphlet insert). This study did not test for interferences.

The IDMS-traceable MDRD, a modified MDRD equation recalculated with serum creatinine measurements that are calibrated to an IDMS traceable enzymatic assay, equation involves the variables as found in equation 2.4 and is favourable as it requires minimal patient information. The Cockcroft-Gault equation has the limitation that it estimates creatinine clearance without being normalised to 1.73 m<sup>2</sup> body surface area, unlike the MDRM equation. The Cockcroft-Gault equation requires accurate body weight which is not always readily available. This equation and the Schwartz equation were both developed using an outdated Jaffe assay for creatinine measurement, which reflects true creatinine. The Schwartz equation involves a *k* constant that differs for age groups and gender. The *k* constant should be determined by the institute against gold standard GFR measurements. *K* constants are also significantly dependant on age and are closely associated with muscle mass. This is difficult to assess in individual patients thus making it a challenging equation to make use of. (Herget-Rosenthal *et al.* 2007)

This study found that the Nova Statsensor® did not measure CKD as accurately as expected when compared against the laboratory reference method, with only a 66.7% sensitivity. This is due to the poor agreement of the direct creatinine measurement and eGFR values between the Statsensor® and the laboratory reference method. These finding are supported Shephard *et al* (2010) where the Statsensor® performance was compared to the Roche enzymatic assay, and performance was found to be lacking. However the CKD staging results displayed a specificity and positive predictive values of 100% therefore demonstrating excellent diagnostic accuracy.

The study's findings of inadequate accuracy are however contradicted in a study conducted by Srihong *et al* (2012) where adequate accuracy was established. This contradiction may be due to the fact that Srihong *et al* conducted their study on *in vitro* stored sample whereas this study was conducted using blood directly sampled from participants in a community setting.

Schnabl *et al* (2010) also stated results that contradict the findings of this study, where they found acceptable correlation between the POCT device results and laboratory reference method results. A possible explanation for these contradicting results could be that Schnabel *et al* used a larger population of 191 renal patients only.

The technical quality of the Statsensor® was satisfactory as it was user friendly, requiring only 1.2 µl of a finger stick blood sample. It also had an incredibly short turn-around-time of 30 seconds. These attributes make the Nova Statsensor® a desirable POCT device for community based screening programmes,

therefore further standardization to IDMS traceability may prove worth the effort and possibly improve the Statsensor® to be used as a diagnostic tool. Having said this, the Nova Statsensor® would be an adequate tool as a screening device for CKD within communities.

The cost of implementation of this POCT device and all the relevant consumables for 1 month will cost the State R 2 771 301.88. This price is mostly unaffordable for governments of developing countries and thus would most likely require a budget plan to be implemented in order to save up for implementation. Other than that, in reality it would be too costly to implement. In addition to the high expenses, there is also the requirement for training personnel on the proper use of the device, this is time consuming and will possibly require additional funding.

Strengths of this study include a wide range of ages used and the study included both male and female participants. The study also included patients with pre-existing conditions that relate to CKD, therefore demonstrating the possible relationship between CKD and several of its risk factors. Another strength of this study was that Nova Biomedical stated that the Nova Statsensor® displayed negligible interference from the following compounds at their respective concentrations: acetaminophen (66.14 µmol/L), ascorbic acid (198.73 mmol/L), bilirubin (256 mmol/L), cholesterol (25.9 mmol/L), creatine (305 mmol/L), glucose (27.75 mmol/L), heparin (120 U/dL), triglyceride (11.3 mmol/L), uric acid (1189.7 mmol/L), and haemoglobin (up to 1.61 mmol/L). This made it unnecessary to test for additional compound interference as it was highly unlikely that non-comparable values were due to interference from other compounds found within the blood sample, this being based on the manufacturer's claims.

The weakness of the study was that there was a very small sample size used. There was also room for pre-analytical errors such as inadequate finger prick sampling, where one forgot to wipe away the first drop of blood as directed by the manufacturer, or venous blood sample being inadequately stored during transportation. Possible areas of improvement include a larger sample size.

This study is unique in that it was conducted in a mixed ancestry population in a community setting under community clinic conditions, where samples were collected and analysed within 24 hours of collection. Whereas the above referenced studies by Schnable *et al* and Srihane *et al* were conducted in a laboratory setting using stored samples collected from hospital renal patients.

### 3.3 Conclusion

Our results demonstrated an overall correlation between Statsensor® and the laboratory reference method that was less than initially anticipated, however the study did find a relatively high prevalence of CKD within the small sample group, thus reiterating the need for community screening programs. This study demonstrates that there is a need to implement infrastructure in order to equip communities with means of running screening programmes in order to combat the ever rising prevalence of CKD and its relevant risk factors. Currently, developing countries cannot afford such infrastructure which opens a window of opportunity for the development of cheaper alternative testing methods that still remain user friendly. The study highlights a need for the establishment and standardization of creatinine and eGFR cut-offs for the many different mixed ancestry populations globally, as there may well be variation between ethnic groups. Our results also demonstrate that eGFR measurement using the MDRD equation is a better method of measuring CKD, rather than using creatinine values alone.

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# Appendices

## APPENDIX A – ETHICS CLEARANCE



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### Approval Notice

#### Response to Modifications- (New Application)

17-Aug-2015  
Erasmus, Rajiv  
RT

**Ethics Reference #: N15/05/045**

**Screening for Chronic Kidney Disease (CKD) in a high risk population using a**

**Title: Point Of Care Instrument for creatinine measurement – a community based  
Study (The Bellville-South Africa Study).**

Dear Prof Rajiv Erasmus,

The **Response to Modifications - (New Application)** received on **30-Jul-2015**, was reviewed by members of **Health Research Ethics Committee 2** via Expedited review procedures on **30-Jul-2015** and was approved.

Please note the following information about your approved research protocol:

Protocol Approval Period: **17-Aug-2015 -17-Aug-2016**

Please remember to use your **protocol number (N15/05/045)** on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

#### **After Ethical Review:**

Please note a template of the progress report is obtainable on [www.sun.ac.za/rds](http://www.sun.ac.za/rds) and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.

Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372

Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

### **Provincial and City of Cape Town Approval**

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health ([healthres@pgwc.gov.za](mailto:healthres@pgwc.gov.za) Tel: +27 21 483 9907) and Dr Helene Visser at City Health ([Helene.Visser@capetown.gov.za](mailto:Helene.Visser@capetown.gov.za) Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.

For standard HREC forms and documents please visit: [www.sun.ac.za/rds](http://www.sun.ac.za/rds)

If you have any questions or need further assistance, please contact the HREC office at 219389207.

### **Included Documents:**

Checklist

CV R Erasmus

Protocol

CV A Zemlin Application form Declaration R Erasmus Declaration A Zemlin  
Declaration M Rensburg MOD\_Protocol Payment form  
Protocol Synopsis

MOD\_Cover letter\_Response to modifications CV M Rensburg

Sincerely,

Mertrude Davids

HREC Coordinator

Health Research Ethics Committee 2



## APPENDIX B – Validation of the NovaStatsensor Creatinine Hospital Meter

Nova Biomedical provided their in-house validation method for the StatSensor-i Creatinine Meter. They made use of 5 predicate devices, that they had previously manufactured and validated, for the comparison studies. Below are the details of said predicate devices.

**Table 1:** Predicate devices used in the comparison studies of the Nova StatSensor-i Creatinine Meter as part of the Nova Biomedical validation study. (*Information provided by Nova Biomedical, as part of their FDA clearance documentation*)

| Characteristic                        | Proposed Nova StatSensor  | Predicate Beckman Synchron LX-20  | Predicate Beckman Synchron LXi 7251   | Predicate Bayer ADVIA 1650                             | Predicate Dade Dimension RXL   | Predicate i-STAT with Creatinine Cartridge                                  |
|---------------------------------------|---|---|---|--|--|---|
| K Number                              | New   | k965240   | k023049   | k990346  | k043546  | k973292   |
| Measuring Range                       | 0.3-12.0 mg/dL  | 0.3-25.0 mg/dL  | 0.3-25.0 mg/dL  | 0.2-37.0 mg/dL   | 0.2-30.0 mg/dL   | 0.2-20.0 mg/dL  |
| Operating Principle                   | Electrochemical biosensor test strip  | Random access processing analyser using a colorimetric, kinetic rate reaction                                       | Random access processing analyser using a colorimetric, kinetic rate reaction                                       | Automated analyser, UV rate reaction                   | Discrete chemistry analyser measuring kinetic rate reaction                        | Electrochemical biosensor cartridge   |
| Intended Use                          | The Nova StatSensor-i Creatinine Hospital Meter System is intended for <i>in vitro</i> diagnostic use by health care professionals for the quantitative measurement of creatinine in capillary, venous, and arterial whole blood. It is indicated for the use in a clinical setting by healthcare professionals as an aid to monitor kidney function. | <i>In vitro</i> diagnostic use by health care professionals for the quantitative measurement of creatinine in serum | <i>In vitro</i> diagnostic use by health care professionals for the quantitative measurement of creatinine in serum | To measure the creatinine levels in serum and urine    | <i>In vitro</i> diagnostic assay for the quantization of creatinine in human serum | For the quantitative determination of creatinine in whole blood.            |
| Sample Type                           | Whole blood   | Serum   | Serum   | Serum  | Serum  | Whole blood   |
| Sample Size                           | 1.2 µL  | 20 µL   | 20 µL   | 2 µL of a 1:5 diluted solution                         | 3 µL diluted serum   | 65 µL   |
| Sample Application                    | Test strip capillary draw   | Automatic sample aspiration from sample cups  | Automatic sample aspiration from sample cups  | Sample Tray 84 samples, positive sample identification | Auto-sampling from vacutainer type tubes or sample cups                            | Test cartridge syringe or capillary fill tube                               |
| Handheld Meter?                       | Yes   | No  | No  | No   | No   | Yes   |
| Meter Calibration                     | Automatic, no Calibration Code  | Automatic, at least every 5 days  | Automatic, at least every 5 days  | Automatic 2-point liquid calibration                   | Automatic  | Automatic calibration with each cartridge                                   |
| Data Storage                          | 1200  | Unlimited using DL2000 data manager   | Unlimited   | 70 000 patient samples                                 | Unlimited, based on available hard disk storage space                              | 5 000 test results  |
| Test Time                             | 30 seconds  | 25 seconds  | 25 seconds  | 20 seconds   | 20 seconds   | 120 seconds   |
| Weight                                | 8.8 oz  | 1 620 lb  | 2 701 lb  | 1 178 lb   | 800 lb   | 18.34 oz  |
| Bar Code Scanner?                     | Yes   | Yes   | Yes   | Yes  | Yes  | Yes   |
| Power Source                          | Rechargeable 3.7 V Lithium battery  | 115 or 220 V outlet   | 115 or 220 V outlet   | 100-240 V main   | 110-130 V outlet   | 2.9 V batteries   |
| Accessories:                          | Charging/Data Upload Station Base Unit  | Data manager  | data manager, automated sample rack system  | Data management system                                 | N/A  | N/A   |
| Controls:                             | Liquid, 3 levels  | 2 levels  | 2 levels  | 3 levels   | 3 levels   | Liquid, 3 levels  |
| Linearity Solutions                   | Liquid, 5 levels  | 5 levels  | 5 levels  | 5 levels   | 5 levels   | Liquid, 5 levels  |
| Test Strip/ Cartridge Active Reagent: | Creatinine amidohydrolase, creatine amindinohydrolase and sarcosine oxidase   | Jaffe rate method (kinetic alkaline picrate)  | Jaffe rate method (kinetic alkaline picrate)  | Enzymatic reaction/alkaline picrate reagent            | Alkaline picrate, colorimetry, creatinine  | Creatinine amidohydrolase, creatine amindinohydrolase and sarcosine oxidase |

### 2.4.1 Analytical Performance

#### i) Precision/Reproducibility

Precision studies were conducted based on Clinical and Laboratory Standards Institute (CLSI) EP5-A2 (*Evaluation of Precision Performance of Quantitative Measurement Methods*).

- Day-to-day precision was evaluated using two meters and two lots of test strips on site of the sponsor. Samples were run in duplicate and all 3 levels of controls were used. Precision testing was run daily for 20 days.

**Table 2:** Results outline of day-to-day precision evaluation.

| Strip Lot | Control Level | Range (mg/dL) | Mean | CV%  | Number of samples | Duration (days) |
|-----------|---------------|---------------|------|------|-------------------|-----------------|
| 6080301   | L1            | 0.8-1.5       | 1.06 | 8.39 | 80                | 20              |
|           | L2            | 1.7-2.5       | 2.09 | 5.80 | 80                | 20              |
|           | L3            | 4.0-7.0       | 6.14 | 3.35 | 80                | 20              |
| 6080401   | L1            | 0.8-1.5       | 1.08 | 8.68 | 80                | 20              |
|           | L2            | 1.7-2.5       | 2.09 | 5.87 | 80                | 20              |
|           | L3            | 4.0-7.0       | 6.11 | 3.59 | 80                | 20              |

- Within-run precision was established using protocol based on the CLSI EP5-A2 and involved two meters and three lots of strips. Three levels of controls were tested in replicates of 20. For each lot of strips tested the controls were prepared separately. In addition, three blood samples with target creatinine concentrations of 0.3-1.5, 2.5-4.5, and 6.0-12.0 mg/dL were prepared.

**Table 3:** Pooled data of within-run precision evaluation

| <b>Blood Samples</b>   |                              |                     |                          |            |                          |
|------------------------|------------------------------|---------------------|--------------------------|------------|--------------------------|
| <b>Strip Lot</b>       | <b>Blood Level (mg/dL)</b>   | <b>Mean (mg/dL)</b> | <b>Ref Value (mg/dL)</b> | <b>CV%</b> | <b>Number of samples</b> |
| 6072601                | 0.3-1.5                      | 0.98                | 0.9                      | 8.73       | 20                       |
|                        | 2.5-4.5                      | 2.43                | 2.6                      | 2.95       | 20                       |
|                        | 6.0-12.0                     | 6.95                | 6.6                      | 4.42       | 20                       |
|                        |                              |                     |                          |            |                          |
| 6072701                | 0.3-1.5                      | 1.11                | 1.0                      | 8.03       | 20                       |
|                        | 2.5-4.5                      | 3.94                | 4.1                      | 3.52       | 20                       |
|                        | 6.0-12.0                     | 10.64               | 9.9                      | 5.16       | 20                       |
|                        |                              |                     |                          |            |                          |
| 6072702                | 0.3-1.5                      | 0.99                | 0.9                      | 9.48       | 20                       |
|                        | 2.5-4.5                      | 3.53                | 3.6                      | 4.77       | 20                       |
|                        | 6.0-12.0                     | 10.23               | 10.0                     | 5.91       | 20                       |
| <b>Control Samples</b> |                              |                     |                          |            |                          |
| <b>Strip Lot</b>       | <b>Control Level (mg/dL)</b> | <b>Mean (mg/dL)</b> | <b>SD</b>                | <b>CV%</b> | <b>Number of samples</b> |
| 6072601                | L1 (0.8-1.5)                 | 1.12                | 0.06                     | 5.27       | 20                       |
|                        | L2 (1.7-2.5)                 | 2.14                | 0.05                     | 2.29       | 20                       |
|                        | L3 (4.0-7.0)                 | 6.16                | 0.30                     | 4.84       | 20                       |
|                        |                              |                     |                          |            |                          |
| 6072701                | L1 (0.8-1.5)                 | 1.13                | 0.08                     | 6.99       | 20                       |
|                        | L2 (1.7-2.5)                 | 3.94                | 0.14                     | 3.52       | 20                       |
|                        | L3 (4.0-7.0)                 | 6.20                | 0.36                     | 5.83       | 20                       |
|                        |                              |                     |                          |            |                          |
| 6072702                | L1 (0.8-1.5)                 | 1.10                | 0.06                     | 5.52       | 20                       |
|                        | L2 (1.7-2.5)                 | 2.16                | 0.05                     | 2.33       | 20                       |
|                        | L3 (4.0-7.0)                 | 6.25                | 0.31                     | 4.91       | 20                       |

The above mentioned protocol was followed while conducting the within-run precision study at three different Point of Care sites, where the tests were carried out by typical Point of Care staff. It was found that there was no significant difference in precision at the various sites.

**Table 4:** Summary of within-run precision results at the three different Point of Care testing sites.

|             | Site 1               |         |          | Site 2               |         |          | Site 3               |         |          |
|-------------|----------------------|---------|----------|----------------------|---------|----------|----------------------|---------|----------|
|             | Target Range (mg/dL) |         |          | Target Range (mg/dL) |         |          | Target Range (mg/dL) |         |          |
|             | 0.5-1.5              | 3.0-5.0 | 8.0-12.0 | 0.5-1.5              | 3.0-5.0 | 8.0-12.0 | 0.5-1.5              | 3.0-5.0 | 8.0-12.0 |
| <b>Mean</b> | 0.84                 | 4.42    | 11.42    | 0.88                 | 4.10    | 11.38    | 0.84                 | 4.53    | 8.15     |
| <b>SD</b>   | 0.08                 | 0.34    | 0.85     | 0.09                 | 0.17    | 0.60     | 0.05                 | 0.13    | 0.26     |
| <b>CV%</b>  | 9.73                 | 7.72    | 7.43     | 10.41                | 4.26    | 5.24     | 5.86                 | 2.77    | 3.25     |

**ii) Linearity/assay reportable range**

The Nova StatSensor-i Creatinine Meter, with a measuring range of 0.3-12.5 mg/dL, was assessed for linearity using the protocol based on the CLSI EP6-A (*Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline Evaluation of Matrix Effects*).

Venous blood samples were prepared by spiking blood with 5 different concentration of creatinine and 3 levels of haematocrit. 2 meters were used where 5 replicates were run at each level. A total of 30 samples were run at each creatinine concentration.

The results obtained were:

$$Y = 1.0068x - 0.0398$$

(2.5)

$$r^2 = 0.9927$$

(2.6)

$$n = 450$$

(2.7)

These results were said to support the claimed linearity range.

**iii) Traceability, Stability, Expected values (controls, calibrators, or methods)**

The Nova StatSensor-i Creatinine controls and linearity solutions values are traceable to the NIST SRM967 Creatinine. Criteria for acceptance between the NIST Creatinine standard and Nova Control solutions are +/-5% (L1), +/-4% (L2), and +/-3 (L3). The expected control range values are as follows: L1=0.8-1.5 mg/dL, L2=1.7-2.5 mg/dL, L3=4.0-7.0 mg/dL. The formal concentrations of the Nova Linearity solutions are as follows: L1=0.30-0.80 mg/dL, L2=0.80-1.50 mg/dL, L3=1.70-2.50 mg/dL, L4=4.00-7.00 mg/dL, L5=7.00-12.00 mg/dL.

Opened Bottle Stability: According to the sponsor, the Linearity and Control solutions are stable for up to 5 weeks after date of opening, when stored at 2-8 °C. It was stated that stability up to 5 weeks is also viable when the solutions are stored at 25 °C. This was demonstrated in real time studies. It was found that all analytes satisfied the sponsor's acceptance criteria of +/-15 %, (for L1) and +/-10 % (for all other levels) deviation from analyte concentration at day 0.

Closed Bottle Stability (Shelf Life): According to the sponsor, the shelf life of the solutions are stable for up to two years from the date of manufacture, when stored at 2-8 °C. All analytes were tested at temperatures of 5, 25, 40, and 50 °C for 9 weeks in order to predict the two year stability, when the analytes are stored at 2-8 °C. The percentage loss of stability was determined in comparison to Day Zero values. The product is considered to be stable when the reported loss is less than or equal to 15 %. All analytes met the sponsor's acceptance criteria, therefore verifying the closed bottle stability claim. Real time studies are still being conducted.

#### **iv) Detection limit**

The Nova StatSensor-i Creatinine Meter detection limit was assessed by following the Nova Biomedical Co.'s protocol, which was developed according to CLSI-EP17-A (*Interference Testing in Clinical Chemistry*). The limitation of Blank (LoB) was determined to be 0.055 g/dL and the Limit of Detection (LoD) was determined to be 0.124 mg/dL.

#### **v) Analytical specificity**

Interference studies were carried out based on the CLSI EP7-P (*Interference Testing in Clinical Chemistry*). The studies involved several compounds which were used to determine if their presence affected the creatinine in whole blood reporting. The studies were carried out where three whole blood samples were prepared with target ranges of 0.5 - 1.5, 2.0 - 4.0, and 6.0 - 10.0 mg/dL. The above mentioned compounds were added in four different concentrations and three samples were run for each of these concentrations. The sponsor defined non-interference as  $\leq 15\%$  for the target range 0.3 - 1.5 mg/dL and  $\leq 10\%$  for the target ranges  $> 1.5$  mg/dL.

The following compounds produced no interference at the relevant concentrations:

Acetaminophen 10 mg/dL, Ascorbic Acid 3.5 mg/dL, Bilirubin 15 mg/dL, Cholesterol 1000 mg/dL, Creatine 4 mg/dL, L-Dopa 0.3 mg/dL, Dopamine 10 mg/dL, Glucose 500 mg/dL, Heparin 120 U/dL, Maltose 100 mg/dL, Triglyceride 1000 mg/dL, Uric Acid 20 mg/dL, and Haemoglobin up to 2.6 g/dL.

Hematocrit studies were conducted in order to determine the effect of varying levels hematocrit on creatinine results that were determined by the Nova StatSensor. Blood samples were prepared and spiked at three different levels of hematocrit, 28, 43, 59 %, and five different creatinine concentrations, 0.3 - 1.5, 1.5 - 3.0, 3.0 - 6.0, 6.0 - 9.0, and 9.0 - 12.0 mg/dL. The sponsor again defined non-interference as  $\leq 15\%$  for the target range 0.3 - 1.5 mg/dL and  $\leq 10\%$  for the target ranges  $> 1.5$  mg/dL. The performance of the Nova StatSensor at hematocrit concentrations of 30 – 60 % were defined as acceptable.

Evaluation of humidity and temperature was conducted using whole blood with three creatinine target concentrations. Nova StatSensor meters were placed in environmental chambers at varying humidity and temperature in order to determine the effects of such environmental conditions on the Nova StatSensor Creatinine device. The sponsor defined non-interference as  $\leq 15\%$  for the target range 0.3 - 1.5 mg/dL and  $\leq 10\%$  for the target ranges  $> 1.5$  mg/dL. The Nova StatSensor met the specified criteria when exposed to a temperature range of 15 – 40 °C and a humidity range of 20 – 80 %.

**vi) Assay cut-off**

Not applicable.

**2.4.2 Comparison studies****i) Method comparison with predictive device**

Five external Point of Care sites were used to carry out method comparisons, where typical Point of Care staff were untrained in the use of the Nova StatSensor device. Whole blood venous, arterial, and capillary samples were collected at each of the sites and compared against the predicate devices listed in Table 4.

The samples were endogenous and compared across the measuring range indicated below. There was no significant difference in results across the various sites.

The results were pooled for venous, arterial, and capillary.

$$Y = 0.9873x + 0.0241$$

(2.8)

$$r^2 = 0.9858$$

(2.9)

$$n = 947$$

(2.10)

$$\text{Range} = 0.3 - 12.9 \text{ mg/dL}$$

(2.11)

**ii) Matrix comparison**

The Nova StatSensor Creatinine Hospital Meter is designed for measurement of creatinine within the matrix of whole blood only. A comparison study was carried out where whole blood samples, venous, arterial, and capillary origins, were compared against serum samples, during method comparison.

Samples were again compared against the predicate devices on whole blood samples. Samples were again endogenous and compared across the measuring range as stated below. It must be noted that these samples were the same samples used for the above method comparison study.

Venous Blood

$$Y = 0.9896x + 0.0244$$

(2.12)

$$r^2 = 0.9783$$

(2.13)

$$n = 722$$

(2.14)

$$\text{Range} = 0.30 - 10.8 \text{ mg/dL}$$

(2.15)

**Arterial Blood**

$$Y = 0.9792 x + 0.0787$$

(2.16)

$$r^2 = 0.9946$$

(2.17)

$$n = 76$$

(2.18)

$$\text{Range} = 0.57 - 12.0 \text{ mg/dL}$$

(2.19)

**Capillary Blood**

$$Y = 0.9851 x + 0.0031$$

(2.20)

$$r^2 = 0.9829$$

(2.21)

$$n = 149$$

(2.22)

$$\text{Range} = 0.40 - 12.9 \text{ mg/dL}$$

(2.23)

**2.4.3 Clinical studies****i) Clinical Sensitivity**

Not applicable.

**ii) Clinical specificity**

Not applicable.

**iii) Other clinical supportive data (when a. and b. are not applicable)**

Not applicable.

**2.4.3 Clinical cut-off**

Not Applicable.

**2.4.4 Expected values/Reference range**

Normal Creatinine value is less than 1.2 mg/dL

*The sponsor referenced: Burtis, Carl A & Ashwood, Edward. 1999. Tietz Textbook of Clinical Chemistry.*

## APPENDIX C- SOPs

### Screening for Chronic Kidney Disease (CKD) in a high risk population using a Point of Care Instrument for creatinine measurement – a community based study (The Bellville South Africa Study)

#### STANDARD OPERATING PROCEDURE 1

**Title:** Procedure for performing quality control testing  
**Analyzer:** NOVA StatSensor-i Creatinine Meter  
**Written by:** Tammy Krige (Dr Megan Rensburg)  
**Date:** 1 June 2016

#### 1. Purpose:

This operational procedure describes the correct way to perform quality control testing on the NOVA StatSensor-i Creatinine Meter using point of care test cartridges to ensure optimum operation. The operational procedures are based on information obtained from the NOVA StatSensor-i Creatinine Meter Quick Reference Guide and the NOVA StatSensor-i Creatinine Control Solution inserts.

#### 2. Scope:

This procedure is aimed at all trained and certified operators of the NOVA StatSensor-i Creatinine Meter. Quality control testing using the NOVA StatSensor-i Creatinine Control should be done to confirm that your NOVA StatSensor-i Creatinine Meter System is working properly and provides reliable results. Only when controls are used routinely and the values obtained are within acceptable ranges can accurate results for patient samples be assured.

#### 3. Reagents, Equipment and Material

- NOVA StatSensor-i Creatinine Meter
- NOVA StatSensor-i Creatinine Test Strips REF 43272
- NOVA StatSensor-i Creatinine Control 1 Solution REF 43921
- NOVA StatSensor-i Creatinine Control 3 Solution REF 43923
- NOVA StatSensor-i Creatinine QC Log Sheet



#### **4. Warnings and Precautions**

- For in vitro diagnostic use.
- Use only the StatSensor Creatinine Control Solution for testing with the Creatinine Meter and Statsensor Creatinine Test Strips.
- Proper handling and disposal methods should be followed in accordance with local, state regulations.
- Use personal protective equipment.
- Do not use the StatSensor-i controls after their expiration date or if they have not been stored in accordance with recommendations.
- Use for only 90 days after first opening.
- Discard the vial if there is evidence of microbial or fungal contamination.
- Do not consume the control solution. If swallowed, seek immediate medical attention.

#### **5. Storage Instructions**

- The expiration date of the kit only applies if the product is stored at 2-8°C in the original container. The expiration date is the last day of the month stated on the outer container and vial label.
- Avoid exposure to direct sunlight and temperatures above 30°C.
- Do not freeze.

##### **Unopened control vials**

- Unopened control vials are stable until expiration date indicated on the vial label when stored refrigerated 2-8°C.

##### **Opened control vials**

- Opened controls stable for 90 days after opening when stored at 2-8 °C.
- It is recommended to note the date of opening and the new expiry date on the vial label.
- Replace the cap immediately after use.
- Always store the control vials refrigerated 2-8°C when not in use, with cap tightly closed.
- Opened control vials should be stored in an upright position.

#### **6. Analyzing a Control**

It is recommended to keep a permanent record of all quality control results on the NOVA StatSensor-i Creatinine QC Log Sheet. The StatSensor-i Creatinine Meter automatically stores the control results in a separate record. Consult the StatSensor-i Creatinine Meter User Manual.

**6.1. Frequency of control testing Controls should be analyzed:**

- Every day prior to study sample collection.
- With each new shipment of StatSensor-i Creatinine test kits.
- With each new lot of StatSensor-i Creatinine test kits.
- At least every 30 days.
- When training new operators in correct use of the StatSensor-i Creatinine test kit and StatSensor-i Creatinine Meter.
- Anytime an unexpected test result is obtained.

**6.2. Test Procedure**

Consult the StatSensor-i Creatinine Meter User Manual for information related to the general operation of the Meter and StatSensor-i Test Strips handling.

- Control material can be used directly from storage at 2 to 8 °C.
- Mix the control material thoroughly by gently inverting the vial several times, avoid bubble formation.
- Wipe away the first drop of control solution using paper towel.
- Insert Test Strip into the Meter and complete set up steps.
- Collect a sample using the StatSensor-i Creatinine Test Strip in the Meter. The sample can be extracted by dropping the solution directly onto the Test Strip.
- Clean the outside of the control vial neck and replace the cap tightly.
- Immediately return the control vial back to the refrigerator after use.

A detailed, illustrated instruction on how to analyze a control sample is provided in the StatSensor-i Creatinine Quick Reference Guide.

**6.3. What do I do if StatSensor-i Creatinine Control results are not within the acceptable range?**

- Do not analyze any patient samples.
- Check the control vial label to make sure it is not expired.
- Ensure that the control has not been used for more than 90 days.
- Verify that the controls and test cartridges have been stored correctly.
- Verify that there is no visual sign of bacterial or fungal growth in the control vial.
- Correct any procedural error. Re-test the control.
- If the control values are still not within acceptable range, repeat the test using a new vial of control. If the control results are still not acceptable, call Technical Support.

**7. References**

1. StatSensor-i Creatinine Hospital Meter. *Nova Biomedical*. Quick Reference Guide.
2. StatSensor-i Creatinine Control package insert.

## STANDARD OPERATING PROCEDURE 2

**Title:** Patient preparation and taking a finger stick sample

**Written by:** RT Erasmus

**Date:** 1 June 2016

**1. Purpose:**

This SOP describes the procedure before a blood sample (finger stick) is taken as well as the procedure for taking a finger stick sample

**2. Scope:**

Though this procedure is aimed at all trained and certified operators of the NOVA StatSensor-i Creatinine POCT Meter but can be applied to any point of care test

**3. Procedure:**

- a. Explain procedure to patient (see below)
- b. **Informed consent** is required for this procedure. Subject should sign the form
- c. **Infection Control:** Gloves should be worn as for handling blood products (see also safety SOP)
  - Safe disposal of lancets – should go into sharps bin.
  - Safe disposal of test strips – should go into clinical waste bin.
  - There should be no blood stained equipment in workstation & the only reason for this is if staff are placing used equipment in workstation following a test.
  - Staff will be **audited** on this so any blood stained equipment should be cleaned or replaced as appropriate.
  - **Handwashing:** To prevent contamination of the blood sample, both nurse & patients hands should be washed in soap & water rinsed & dried thoroughly. Or alternatively plain tap water.
  - **Do not use alcohol wipes/gel as can cause inaccurate results.**
  - **There should be no** food, fruit juice, newspaper print, perfumes, hand creams, Glossy magazines (paper contains glycerine), hairspray, hair gel.

**d. Taking a blood sample**

**Supplies:** The following should have been assembled on a tray prior to taking the sample:

- Microtainers
- Retractable lancets
- Gauze pad or cotton ball
- Tape (Band Aid)
- Sharps Container

### **Lancets**

Various devices for skin puncture are available. Refer to the manufacturer's directions for optimum performance.

**Patient identification-** Testing personnel must positively identify the patient that is being drawn. The following steps ensure patient identification:

- a. Ask the patient to state their full name, including the spelling of an unusual name. If the patient is very young, ask his/her parents or guardian to state the name and/or the correct spelling.
- b. Compare the name with that on the patient chart you have.

Joint Commission: "Use at least two ways to identify patients. For example, use the patient's name and date of birth. This is done to make sure that each patient gets the correct medicine and treatment."

**Fasting Status:** Determine whether the patient has fasted (if necessary) and record if necessary (notes column). Some tests require the patient to fast or to eliminate certain foods from the diet before the blood drawing. Time and diet restrictions vary according to the tests. Such restrictions are needed to ensure accurate results.

**Allergies:** Verify that the patient is free of latex allergies if a latex glove is worn

**Wash your hands, dry them and put on gloves.**

### **Subject Preparation**

#### **Reassuring the Patient**

Testing personnel must gain the patient's confidence and assure him that although the puncture will be slightly painful, it will be of short duration. Patients should never be told that "this will not hurt," and they should be told when the lancet enters the skin so as to avoid fright.

Ensure subject is comfortable and that the subject is ready to proceed. Advise subject not to withdraw their hand suddenly when the lancet is applied. Ensure subject's hand is warm. Subject may wash hands using warm water or exercise hand to increase blood flow.

Position subject as either sitting or lying down. The hand from which a specimen is to be collected should be below the level of a subject's heart.

**To collect any blood spillage,** place small disposable sheet underneath subject's forearm on the side chosen for taking fingerprick specimen. Place lancets and plastic mini-pipette on clean dry surface next to subject's hand with appropriate biohazard sharps disposal bin within easy reach.

## Procedure for Fingerstick

**Sites for Lancing:** Avoid using index finger & thumb as they are used for fine motor movements. Lance sides of fingers – not pad or tip. Explain if patient was to become visually impaired using the pad could affect their ability to read Braille & more painful in pad. Lance no lower than nail bed avoiding edge of nail. Always rotate sites to prevent sore areas.

- a. Clean the chosen puncture site and allow the site to thoroughly dry. Perform the puncture on the centre of the palmar surface the finger- not at the side or tip of the finger (check), because the tissue on the side and tip of the finger is about half as thick as the tissue in the centre of the finger.
- b. The middle finger and ring finger are the preferred site. The fifth finger must not be punctured, because the skin is too thin. Avoid a finger that is cold, cyanotic (blue), swollen, or inflamed.
- c. Position subject so that the finger is steady and supported in a comfortable position.
- d. With your thumb and index finger, grasp the patient's finger between 6 -8 cm from the tip of the finger.
- e. With your other hand, hold the sides of the patient's finger.
- f. Moving your supporting hand toward the tip of the patient's finger. Apply a massaging motion to the fleshy portion of the finger.
- g. Repeat this massaging process five or six times.
- h. Clean the chosen puncture site and allow the site to thoroughly dry.
- i. Select the appropriate retractable lancet, position it above the selected site, and activate. Discard of used lancet in approved puncture-resistant sharps container.
- j. Puncture sites should be oriented perpendicular to the lines of the fingerprint (across the fingerprint).
- k. If the cut is made across the fingerprints and the area has been wiped dry, the blood should well up into a large rounded drop. (If the cut has been made along the lines of the fingerprint, the blood will stream down the finger).
- l. After the chosen site has been prepared and punctured, the first drop of blood should be wiped away with a gauze pad, since the first drop is most likely to contain excess tissue fluid. Discard of gauze in a biohazard container.
- m. If blood does not flow freely, increase blood flow by holding the finger downward and applying gentle continuous pressure above the puncture site. Do not massage the area since this may contaminate the blood sample with tissue fluid.
- n. Wipe away first drop of blood with sterile gauze pad. Hold finger lower than elbow and apply gentle, intermittent pressure.
- o. If blood does not flow easily after gentle pressure, make another puncture using a new sterile lancet.
- p. Fill the testing device as needed by gently scooping up the drops of blood and allowing them to roll into the container.
- q. Drops of blood should be allowed to flow freely.
- r. After blood has been collected from the patient's finger, place a piece of gauze on the site and apply gentle pressure to stop the blood flow.

- s. Apply tape to puncture site after bleeding has stopped. Subject may also be asked to apply gentle pressure
- t. Remove gloves and wash hands.
- u. Dispose of lancet immediately in biohazard sharps container. Discard gloves, , and other used materials in their appropriate containers.

### **Additional Considerations**

Blood should not be taken from swollen or previously punctured site, because accumulated tissue fluid will contaminate the blood specimen.

### **References**

- NCCLS Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture; Approved Standard- Fourth Edition, September 1999
- NCCLS Specimen Collection 1989. 6.14-3.
- Blumenfeld, TA: Clinical Application of Microchemistry. In Werner M(ed): Micro-Techniques for the Clinical Laboratory: Concept and Application, pp 1-15. New York, John Wiley & Sons, 1976.
- Blumenfeld TA, Hertelendy WG, Ford SH: Simultaneously Obtained Skin Puncture Serum, Kin Puncture Plasma and Venous Serum compared and Effects of Warming the Skin Before Puncture. Clinical Chemistry 23: 1705, 1977.
- Alreja G, Setia N, Nichols J, Pantanowitz L. Reducing patient identification errors related to glucose point- of-care testing. J Pathol Inform 2011; 2: 22 [<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3097526/>]

### STANDARD OPERATING PROCEDURE 3

**Title:** Storage and Stability  
**Analyzer:** NOVA StatSensor-i Creatinine Meter  
**Written by:** Tammy Krige (Dr Megan Rensburg)  
**Date:** 1 June 2016

#### 8. Purpose:

This operational procedure describes the storage and stability of the NOVA StatSensor-i Creatinine Meter point of care Test Strips to ensure optimum operation. The operational procedures are based on information obtained from the NOVA StatSensor-i Creatinine Controls package inserts.

#### 9. Scope:

This procedure is aimed at all trained and certified operators of the NOVA StatSensor-i Creatinine Meter.

#### 10. Reagents, Equipment and Material

- NOVA StatSensor-i Creatinine Meter
- NOVA StatSensor-i Creatinine Test Strips REF 43272
- NOVA StatSensor-i Creatinine Control 1 REF 43921
- NOVA StatSensor-i Creatinine Control 3 REF 43923

#### Storage and Stability

##### 10.1. StatSensor-i Creatinine Meter

###### **Desktop/handheld use**

- Store the NOVA StatSensor-i Creatinine Meter on a dry, clean, stable and horizontal surface.
- Empty the Test Strip chamber for storage.
- Store away from:
  - Condensing humidity and water
  - Heat and large temperature variations
  - Direct sunlight
  - Vibrations (e.g. from centrifuges and dishwashers)

## Transportation

- Store the NOVA StatSensor-i Creatinine Meter in the original packaging.
- Ensure the StatSensor-i Creatinine Meter unit includes:
  - StatSensor-i Creatinine Meter
  - Power cable and cord adapter
  - Quick Guides for the available StatSensor-i Creatinine Tests
  - User Manual

### 10.2. StatSensor-i Creatinine Test Strips REF 43272

#### Refrigerated Storage: 2- 8 °C

- The StatSensor-i Creatinine Test Strips are stable until the expiry date (12 months) only when stored refrigerated in original sealed plastic containers and unopened.
- The expiry date is the last day of the month stated on the container and packaging box.
- The StatSensor-i Creatinine Test Strips must reach an operating ROOM temperature of +/- 25 °C before use.
- Upon removal from refrigerated storage, leave the Test Strips in the capped container, preferably overnight.
- Do not freeze.

#### Room Temperature Storage: 15 – 25 °C

- StatSensor-i Creatinine Test Strips are stable for up to 90 days or until the expiration date, whichever comes first.
- Once opened, keep tightly sealed in original storage container.
- Avoid exposure to direct sunlight.

### 10.3. StatSensor-i Creatinine Control Solutions REF 43921 and REF 43923

- The expiration date of the kit only applies if the product is stored at 2-8 °C in the original container.
- The expiration date is the last day of the month stated on the outer container and vial label.
- Avoid exposure to direct sunlight and temperatures above 30 °C.
- Do not freeze.
- Can be used for running QC straight out of the fridge.

#### Unopened control vials

- Unopened control vials are stable until expiration date (period of up to 12 months) indicated on the vial label when stored refrigerated 2-8 °C.



### **Opened control vials**

- Opened control vials are stable for 90 days when stored refrigerated 2-8 °C.
- It is recommended to note the date of opening and the new expiry date on the vial label.
- Replace the cap tightly immediately after use.
- Always store the control vials refrigerated 2-8 °C when not in use.
- Opened control vials should be stored in an upright position.

### **11. References**

1. NOVA StatSensor-i Creatinine Hospital Meter. *Nova Biomedical*. Quick Reference Guide.

## STANDARD OPERATING PROCEDURE 4

**Title:** Troubleshooting  
**Analyzer:** NOVA StatSensor-i Creatinine Meter  
**Written by:** Tammy Krige (Dr Megan Rensburg)  
**Date:** 1 June 2016

### 1. Purpose:

This operational procedure provides information on how to troubleshoot when errors codes are displayed on the **NOVA StatSensor-i Creatinine Meter Analyzer** whilst operating the instrument.

### 2. Scope:

This procedure is aimed at all trained and certified operators of the NOVA StatSensor-i Creatinine Meter.

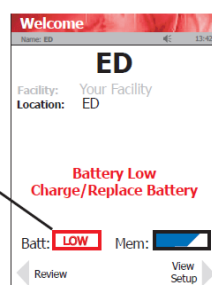
### 3. TROUBLESHOOTING ERROR CODES

An Error Code message with a description of the problem will be on display if an operational or system problem occurs.

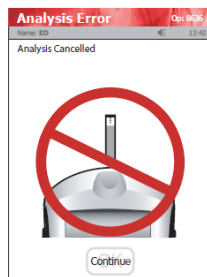
A few examples of an Error code:

1. **Battery Low** - Change the battery or place the meter onto the Charging Station.

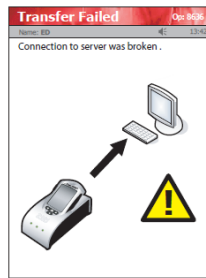
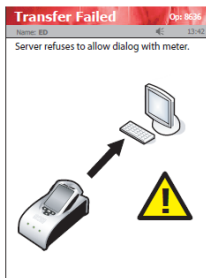
Battery Low Icon



- 2. Analysis Cancelled** - Test Strip Was Removed. The test has been cancelled, repeat the test with a new test strip. Leave the test strip in place until the result is displayed on the screen.



- 7. Transfer Failed** - Server refuses to allow dialog with meter, or Connection to server was broken. Please check the network settings, status of your network, or contact your administrator for assistance.



Refer to the NOVA StatSensor-i Creatinine Meter Quick Reference Guide Information codes and troubleshooting page 30-37.

If you are still encountering error codes after implementing the corrective action contact the following persons:

- NOVA (Brendan Freitag - Copyright [REDACTED])
- Project leaders
  - Prof RT Erasmus [REDACTED]
  - Dr M A Rensburg [REDACTED]

#### 4. References

1. Quick Reference Guide. *nova biomedical*. Statsensor-i Creatinine HOspital Meter.

## STANDARD OPERATING PROCEDURE 5

**Title:** Cleaning and Maintenance Procedure

**Analyzer:** NOVA StatSensor-i Creatinine Meter

**Written by:** Tammy Krige (Dr Megan Rensburg)

**Date:** 1 June 2016

### 5. Purpose:

This operational procedure describes how to maintain the NOVA StatSensor-i Creatinine Meter to ensure optimum operation. The operational procedures are based on information obtained from the NOVA StatSensor-i Creatinine Meter User Manual.

### 6. Scope:

This procedure is aimed at all trained and certified operators of the NOVA StatSensor-i Creatinine Meter.

### 7. Reagents, Equipment, Material

- NOVA StatSensor-i Creatinine Meter.
- Disinfectant

### 8. Maintenance Procedures:

No maintenance of NOVA StatSensor-i Creatinine Meter is required other than cleaning the exterior, as well as charging and changing the battery.

#### **Cleaning the exterior**

- Cleaning the exterior of the NOVA StatSensor-i Creatinine Meter should be performed whenever necessary. Most spills and stains can be removed with water or a mild detergent. Power off the Meter while cleaning.
- Clean the outside of the Analyzer and the touch display with a clean, non-abrasive cloth dampened in water, or detergent, to remove any spills or dirt marks.
- To disinfect the exterior of the instrument, use any of the following: Dilute bleach (10% solution of household bleach, Sodium Hypochlorite), 70% isopropyl alcohol. Avoid benzene and strong acids.
- Using any of the above, dampen the cloth and wipe down the Meter surfaces.
- Wipe dry immediately.
- Power on the Meter.

**NOTE:** Never immerse the device. Never spray the device with disinfectant.

## **Charging and changing the battery pack:**

### **Docking/Charging Station:**

- When the meter is not in use, store it in the docking/charging station. This enables the meter to remain fully charged and connected to the computer network.
- When the battery is LOW symbol displays on the screen, place the device into the docking/charging station. If you have a spare fully charged battery, change with the low battery.

**NOTE:** The left, green light is on if the station is connected to the network. The middle, green light is on if data is transferring. The right light is green for fully charged, or yellow for charging.

### **Changing the battery:**

If you have a spare, fully charged battery it can be changed with the drained battery to allow for continuous use of the Meter.

**WARNING:** Replace the drained battery with NOVA P/N 46827 ONLY. Using another battery may present a risk of fire or explosion. If discarding, dispose of battery promptly. Keep away from children.

### **Instructions:**

- Press the power button to enter “Sleep Mode”, allowing for 20 seconds to change the battery without losing date/time settings. (If it takes longer than 20 seconds of changing the battery, then power up the Meter again and re - login, and set date and times.
- To release battery cover, push down on the two cover latches. Remove the cover.
- Push down on the battery latch to remove the drained battery.
- Replace with a fully charged battery by insertion from bottom first, then push in the top of the battery.
- Replace the battery cover and dock the meter into the charging station before use.
- Replace the drained battery into the separate charging station.

## **9. Safety precautions**

Recommended to use gloves when handling any potentially biohazardous material and adherence to general principles of health and safety precautions should be followed.

## **10. References**

1. StatSensor-i Creatinine Hospital Meter. *Nova Biomedical*. Quick Reference Guide.

## STANDARD OPERATING PROCEDURE 6

**Title:** Safety Procedure

**Written by:** RT Erasmus

**Date:** 1 June 2016

### 1. Purpose:

To describe safety procedures to be carried out before and after taking blood samples

### 2. Scope:

This procedure is aimed at all trained and certified operators of the Nove StatSensor-i Creatinine Meter. It also applies to all Point of Care Instruments and Collecting Devices

### 3. General Information:

POC devices pose a risk of transmitting infectious organisms  
Reagents and carriers besides the devices can also transmit infectious organisms.

### 4. Health and safety :

Universal precautions must be observed when collecting blood specimens. All samples should be handled as potentially infectious for HIV, Hep B and other pathogens.

#### **Check the following:**

Hand washing facilities and soap should be available

Disinfectants should be available for any spillage

Eye decontamination station should be available

#### **GLOVES SHOULD ALWAYS BE WORN WHEN HANDLING BLOOD SPECIMENS**

- i. Prepare the equipment on the clinical workspace before the subject enters the room.
- ii. Ensure the clinical waste bin and sharps bin are readily available for point of generation disposal.
- iii. Wash hands and don gloves and goggles
- iv. Perform procedure as per clinical guidelines
- v. Dispose of sharps and clinical waste at point of generation into properly labelled or colour-coded sharps containers and biohazard trash bags and bins.
- vi. Eating, drinking, or applying makeup in areas where specimens are collected and where testing is being performed is prohibited
- vii. Instrument may require to be disinfected (check guidelines)

**Patient Safety**

- Reassure patient.
- Explain procedure
- Do not share lancets between patients
- Check for bleeding and ensure that bleeding has stopped before patient leaves the room
- All sharps and strips etc to be disposed before the next patient presents
- Disinfect area if there is any contact with blood products

**CONSULT THE FOLLOWING**

- A written plan for exposure control, including post-exposure evaluation and follow-up for the employee in the event of an "exposure incident;"
- Use of Universal Precautions, an approach to infection control in which all human blood and certain human body fluids are treated as if known to be infectious for HIV, hepatitis B virus, hepatitis C virus, and other blood borne pathogens. Universal Precautions is one component of Standard Precautions, a broader approach designed to reduce the risk for transmission of microorganisms from both recognized and unrecognized sources of infection in hospitals